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PROCEEDINGS OF A

CONFERENCE ON THEORETICAL BIOLOGY

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Proceedings of a CONFERENCE ON THEORETICAL BIOLOGY

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under the sponsorship of the National Aeronautics and Space Administration
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PREFACE

In 1962 the Space Science Board, National Academy of Science, held a "Space Science Summer Study" for the National Aeronautics and Space Administration (NASA). At this summer study the majority of the members of a "Biology Working Group" recommended that the National Aeronautics and Space Administration make special attempts to exploit opportunities for the advancement of theoretical biology. In order to stimulate interest and thinking in biological theory and to gain for theoretical biology a recognized standing among scientific disciplines, three methods of approach were suggested:

1. NASA can consciously encourage theoretical approaches to biological problems when these appear in individual proposals. This would amount to increased emphasis on theoretical work as a criterion in evaluating proposals.

2. Conferences, symposia, or workshops can be set up periodically to redefine problems and in other ways fulfill the usual requirements of scientific communication. Such meetings would be different only in that they would be almost entirely theory oriented. NASA might share sponsorship with other agencies.

3. In some instances a continuing theoretical group could be established at a university or, possibly, at a NASA center like Ames Research Center. Such a group could address itself to the problem of identifying opportunities for theoretical developments in biology and. . .would do much to stimulate interest among biologists and theoreticians of many kinds in the theoretical opportunities which exist in modern biology.

To initiate a program in theoretical biology, a "Committee to Advise on the Status and Future of Theoretical Biology in the United States" was convened October 30, 1962, in Princeton, New Jersey. The members were Dr. Ernest C. Pollard, Pennsylvania State College, Chairman; the late Dr. Henry Quastler, Brookhaven National Laboratory; Dr. James F. Danielli, State University of New York at Buffalo; Dr. Harold J. Morowitz, Yale University; and Dr. Joseph Engelberg, University of Kentucky. Observers attending were Dr. John R. Totter, Atomic Energy Commission; Dr. James Liverman, Atomic Energy Commission; and Dr. George J. Jacobs, National Aeronautics and Space Administration.

To fulfill the suggested means for instituting theoretical biology activities, this committee proposed that a series of conferences be held, a summer institute be considered, and professorships, fellowships, and centers for theoretical biology be established at universities. To begin activities, a general conference of not more than 35 active participants was suggested. This conference should have two main objectives: (a) a general scientific meeting and (b) a formal business meeting of the participants for recommendations concerning the development of the discipline and also concerning specific activities and actions to be taken. In accordance with this plan, the present conference was convened.

This conference was organized by the American Institute of Biological Sciences' (AIBS) Interdisciplinary Conference Program under the management of Dr. Frank Fremont-Smith and his staff—Mrs. Purcell, Miss Gordon, and Mrs. Swanson. Their cooperation, efficiency, and efforts in making the mechanics of this conference successful and the participants comfortable are gratefully acknowledged.

Editorial credit belongs, of course, to all the participants, as well as to Dr. Fremont-Smith's staff who accumulated the data until the termination of the AIBS Interdisciplinary Conference Program. Dr. James F. Danielli and his staff aided tremendously in editing many facets of the scientific discussion. However, the editor assumes responsibility for any editorial errors.

George J. Jacobs
Editor

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GENERAL INTRODUCTION

The American Institute of Biological Sciences-NASA Conference on Theoretical Biology convened in the Palmer Room of the Nassau Inn, Princeton, New Jersey, Friday evening, November 22, 1963, at 8:30 p.m. with Dr. James F. Danielli, chairman of the conference, presiding.

DANIELLI: We shall begin this conference by asking Dr. Orr E. Reynolds to tell us how he looks on this meeting, and Dr. Frank Fremont-Smith will tell us how we should behave. Then we shall pass around the table and, in view of the fact that not everybody has previously met everybody else, we shall ask each individual to say in a few words what sort of person he is and describe in 2 minutes, at the most, how he is going to make a fundamental contribution to the synthetic cell or any other problem that may come up in the course of the next few days.

FREMONT-SMITH: Or perhaps why he is interested in coming to the conference.

DANIELLI: Or, alternately, criticize the tactics which were used in getting him to attend. Dr. Reynolds?

REYNOLDS: I learned really only this evening that I was expected to make any comments, and I do not know exactly what I am expected to comment on.

I suppose the reason I am in this position is that I had something to do with stimulating the initiation of events that led to the conference. That fact was occasioned by my responsibilities in NASA, so I will tell you my personal reasons for feeling that the subject of this conference (the general topic, theoretical biology) has a special importance to NASA. I do this not as a theoretical biologist, or even as a biologist, or a scientist at all, but as sort of a science fan because I think that is all I can lay claim to in this specific instance.

The logic is really very simple. It seems to me that the process of doing scientific work in space, certainly biological work in space, is an extremely expensive business with a parsimony of data being almost the certain outcome. In other words, we will not be able, within the immediately foreseeable future, to rely on a large volume of data to give us a large number of statistics or to rely upon an empirical observation with large numbers of data in order to get results on which we can depend to advance our understanding of science or of biology.

The only option that appears to me to be available in such a circumstance is to make experiments as meaningful as possible so that we can place reliance on a smaller amount of data; and the biggest weakness in biology from that point of view—at lease the way I look at it—is the absence of very much theory. I am not implying in any way a criticism of biologists for not having developed more theory. I think biology is a very difficult discipline in which to develop theory, but it seems to me that the worthwhileness of biological experiments in space is very likely to depend on the theoretical soundness of the experiments that are done.

I should like to give a little bit of history as to how this belief led to this event. Shortly after I came with NASA in March 1962, there was a Space Science Summer Study called in Iowa City under the auspices of the Space Science Board, and I was asked to attend as a representative of NASA. The biological sessions of the study were chaired by Dr. Allan Brown,

now at the University of Pennsylvania, and cochaired by Dr. Colin Pittendrigh, who is here. In the course of that meeting, I was trying to determine whether I could achieve any agreement in my own thoughts concerning the importance of the development of theory as an essential part of NASA's biology program. One person that I found who agreed (quite heartily, I thought) was Dr. Pollard. At that meeting as a sort of corridor discussion, rather than in the main sessions, with Dr. Liverman from the Atomic Energy Commission (AEC), Dr. Pollard and I discussed the idea of meeting again to talk about what might be done toward developing theoretical biology in the interests of NASA and AEC. We thought our interests were confluent.

Subsequent to that, Dr. Liverman talked with Dr. John Totter of AEC who, I think, is here also. I talked with Dr. George Jacobs, who is also here; and Dr. Pollard talked with Dr. Danielli. There followed subsequently a series of meetings—large and small, formal and informal—which resulted in the present session.

I, for one, am extremely gratified that it was possible to get such a distinguished group of people together at this meeting. I have high confidence that it will, in the long run, be most beneficial, not only to NASA but also to the development of science in general.

DANIELLI: I guess that covers a lot of ground and provides a basis for this meeting. We are beginning to see what is expected of us, if I may say so. And after Dr. Fremont-Smith has told us a little more of what is expected from us, we shall continue. Thank you very much.

As you can see, this is the first relatively large meeting that has come out of the process which we have just had described for us. It is intended as an experiment. It is also intended that we should participate in a general discussion, a so-called business meeting at which everybody here who cares to come (and we hope you will all come) will be asked to put forward his ideas as to what should be done to promote the development of theoretical work in biology.

This particular series of discussions will be run as informally as is possible while still preserving continuity of thought. In other words, chaos is eliminated as a possible mode of conducting the meeting, not because order does not sometimes come out of chaos, but because, in thinking of the stenographic task of reporting chaos, I fear we shall need to limit the range of topics under discussion simultaneously, at any rate. Is that right, Dr. Fremont-Smith?

FREMONT-SMITH: I think it is exactly right. It all depends on your definition of chaos. I think we need an appropriate amount of chaos in order to get some good thoughts.

DANIELLI: All right, we shall accept you as the chaotic regulator.

FREMONT-SMITH: I should say this about the record. Everything you say will be taken down unless it is so outrageous that you really want it off the record; then you will ask Mrs. Swanson, the stenotypist, to raise her hands so that you can see that she does not even wiggle her fingers. However, we beg you not to do so, unless it is a really terrible thing you are going to say, because you will all have a chance to take anything off the record before it is published. We find very often that what you think ought to be off the record turns out to be quite recordable, and you may be very glad to have it on the record.

DANIELLI: What happens if Dr. Pittendrigh thinks what I say is so outrageous that it ought to come off the record?

FREMONT-SMITH: He has a private talk with you, and, unless you agree with him, it stays in the record.

DANIELLI: Should Dr. Piotr Slonimski succeed in getting here, I think that he should be asked to open the discussion on the specificity of organelles. I think we can assume that that will happen; however, Dr. Slonimski will not be here until tomorrow.

The first thing on Sunday morning we shall have some extension of Saturday's discussion, which will be opened by Dr. Sidney W. Fox, who has also provided you with some matter to read beforehand, if you care to do so.

If there are any questions you would like to ask about the program, Dr. Pollard and the rest of us who participated in this organization will do our best to answer the questions. The discussion leaders are not expected to do more than just start a discussion going. Are there any questions you would like to ask me right at this stage or that you would like to ask anybody who has been organizing this meeting?

Dr. Fremont-Smith, are you going to tell us how we should behave at this meeting?

FREMONT-SMITH: I will be delighted to say a few more words. We were very happy to be asked by NASA to help organize this conference, because in the AIBS Interdisciplinary Conference Program we spend a great deal of time (and previously when I was in the Macy Foundation for some 20 years) in studying the process of interdisciplinary communication and trying to set up opportunities for discussion in depth across disciplinary lines. I think most people feel that they have no problems communicating with other people if the other people would only listen and understand them; they do not, however, think that some of the things the others are telling them are really worth paying much attention to and do not pay very much attention to them sometimes.

I want to emphasize the difference between this kind of meeting (and Dr. Danielli has already emphasized it) and the standard scientific meeting—the meeting at which there is a series of papers read at the group, one after another and then, if any discussion really gets going, the chairman invariably has to say: "I am sorry to have to interrupt this fascinating, most important discussion, but we are 20 minutes behind in our agenda and I must call on Professor So-and-So to make another speech at you."

This is what we are trying to get away from. Most of you have learned that there are very important things that do happen of a communicative type at the standard scientific meeting, but they do not take place in the meeting room. Dr. Reynolds spoke about it. He said it was not at a special session but in a corridor conversation that this happened, and this is often true. The people who know their way around go into the corridors, into people's rooms and into the bar to have bull sessions in which most important decisions are made and very important communication takes place. It happens to be usually somewhat limited to the group involved, which may be three or four or five. It is not shared by others and it is never recorded, which may have some advantages but also some disadvantages.

After really spending time and thought on this, we decided that it ought to be possible to reverse the field and to bring that which takes place in the corridors, in people's rooms, and in the bar, into the conference room. And if anybody wants to make a speech, we send him to the bar—and that does not work too badly either.

I would like to take another few minutes to contrast a speech with a conversation. I think that a speech like the one I am now making, I am sorry to say, is a progressive frustration to everybody who is listening to it. Every time an idea comes up you have to repress it, unless you interrupt (which I hope you will not hesitate to do), so that what you get is serial frustration, serial repression of any ideas, doubts, and questions. Your mind is repressed right straight through the speech. This is why people get exhausted at scientific meetings. They cannot act or react unless they go out into the corridors.

Conversation is a feedback mechanism in which those who participate are constantly correcting one another, interrupting with gesture or word, reinforcing or supporting. The result is that they keep each other on the same wavelength, and if any serious doubt arises in

the mind of one or another, he expresses it, and the question can be clarified. I feel that this is the natural human way, this is what society has developed as its process of communication, only we have become so standardized in our scientific meetings that we practically eliminate this. We have become so conditioned—and this is the danger of my making a speech like this—that if we allow the first person at the meeting to make a speech, everybody subsides into the position in which he either sets up his resistance to being frustrated or he thinks about something else.

What I hope we will all do is to have the courage of our ignorance to say comfortably: "Please explain what you are talking about," when somebody says something that you do not understand, instead of feeling that it is somehow a disgrace which you should not admit. If you will only come out and say it, you will be surprised how many people around the table will nod and say, "I am glad you asked that question, because I did not understand either." It is only by so interrupting and making sure that you do understand what is being said, what is the intent, what are the controls, etc., as the proceedings go along that the group can keep with it. And unless the group keeps with it, the group cannot contribute to whatever is being said or explored.

REYNOLDS: May I interrupt? I wanted to give you an example of a problem of the vocabularies. One of my bosses is an engineer. He was having trouble with a problem in biology that he was supposed to explain to somebody else, and he asked me to write it out for him. Then he thought a little about it and he said: "But do not put it in jargon—I mean, put it in engineering jargon."

FREMONT-SMITH: Ideas have got to stand on their hind legs. The ideas that come from the youngest or the oldest are of equal value and have to be looked upon as what value they have as ideas rather than whether they represent something that somebody has expressed; and too many of us are attached to our previous concepts and viewpoints or, even worse, to the viewpoint of our teachers and father figures. These are all right, provided they have their own validity, but they are of no value merely because Claude Bernard or someone else stated them. I think it is very important that we give full respect and full critique to anybody's ideas.

I would like to conclude by giving you a remarkable bit of insight—scientific insight—that Gertrude Stein revealed on her deathbed. Alice Toklas was there, and Gertrude Stein was suffering. She kept crying out to Alice, "Alice, Alice, what is the answer? What is the answer?"

Alice did not know how she could reply, but finally she realized that these were Gertrude's last moments and she had better speak the truth. She finally said, "Gertrude, there is no answer."

"If no answer," said Gertrude, "then what is the question?"

I think this is it in theoretical biology: What is the question? Gentlemen, I am through.

DANIELLI: I do not think we will allow anybody to cross-examine you about your arguments. If they wanted to do that, they should have interrupted, as you pointed out at the beginning of the meeting. They can do this later.

What I will do now is ask Dr. Pollard to give a model demonstration as to how a member of this meeting should introduce himself to the others.

POLLARD: I will tell you what my background is: it is as a physicist. Like a great many physicists, I became frustrated with physics because it seemed to tend toward detail too much. I listened to a very fine talk yesterday at a physics colloquium in which I heard to six significant figures, all about sodium bromide, and it seems to me that biology has more to offer than that.

I am convinced that the advance made in physical science was a mixture of theoretical and experimental advance. I am convinced it was made in an area which I believe to be harder than that of living systems. As a result of the intense, skilled effort it contained, there was an emerging of original thinking. I think if we apply theoretical thinking to biology we will have this combined approach, and we will take this "easy" subject and make something out of it very fast.

DANIELLI: Now we will ask Dr. Jacobs to give us a second model introduction.

JACOBS: I am a biologist with laboratory experience in hematology and radiation biology. For the past 5 years I have been in administration in space biology at NASA. I am now Chief of Physical Biology with the Bioscience Programs Office. Physical biology covers a broad spectrum of endeavors including "general activities" such as this conference. My aim and, I must admit, my prime interest, is to aid in the development and acceptance of theoretical biology by the scientific community. This conference I feel is a major step in this direction.

DANIELLI: I think we might work around the table. Mr. Leigh, would you like to fire away?

LEIGH: My name is Egbert Leigh. I am by preference a naturalist, by undergraduate training a mathematician. I am interested in two classes of questions. One is: What particular mathematical formalisms are most useful in handling biological questions? I think there is a common mathematical language which will work equally well in certain kinds of physiological problems, such as biological clocks, and in ecology. I am also interested in problems of a more concrete nature—the possible contributions of paleoecology to a more exact theory of evolution and the possible uses of molecular biology considerations in evolution.

PITTENDRIGH: My name is Colin Pittendrigh. I think of myself as a general biologist. I have strong interests in evolutionary theory, and I am currently interested in daily biorhythms, sometimes known as biological clocks. I was persuaded to join the space program by some of my friends.

BRUCH: I am Carl Bruch. I am with the Bioscience Programs Office of NASA. The aspects that I am associated with are the exobiology and the sterilization programs. I arrived at NASA last August. My past experience was 5 years in industry as an industrial microbiologist. My interests in microbiology centered on microbial fermentations and sterilization. After I received my doctorate, I felt I was lacking in knowledge of microbial sterilization and went into this field and did research for 4 years. During that time I became acquainted with NASA's Bioscience Programs. My last year in industry was in yeast fermentation studies.

Although my training in microbiology followed classical lines—i.e., microbiology as a descriptive and taxonomic science—I fortunately took a minor in fermentation biochemistry. I realize that micro-organisms have to work within the framework of chemical and physical laws. I am attending this conference to learn, and this process started this evening by sitting next to one of Dr. Morowitz' graduate students at dinner.

HOFFMAN: My name is Donald Hoffman. I am a graduate student in the Department of Molecular Biology and Biophysics at Yale. My background was in physics before going there. I think it would be a little presumptuous to say anything about my fundamental contributions because they have been nil so far.

I am interested in the mode of thinking and philosophy of theoretical physics as applied to biology, rather than a blank application of the laws of physics. Further, I am interested in the development of biological models and biological systems theory.

LANGELAND: My name is Tor Langeland. I am in the Physics Institute in Oslo, Norway, in a biophysics group there. At present I am a visitor in the Department of Molecular Biology and

Biophysics at Yale University. My education was in physics, and I have done some work on ultraviolet and ionizing radiation action on enzymes. In theoretical biology I am particularly interested in the application of thermodynamics to biology.

ATWOOD: My name is Kim Atwood. I have a mixed background. I thought that I would be interested in what the other people here had to say and, not being an authority, I hoped that Dr. Fremont-Smith would pardon a few sarcastic and destructive remarks. They seem to be so much easier to think of than the constructive ones.

I had the strange experience of participating in a meeting that bore a resemblance to the proceedings of this one, as I anticipate them, which was held last winter at Ohio Wesleyan. The subject was "The Prospects for the Control of Human Evolution." To give you an idea of the flavor of the meeting, we considered the prospects of when complete control would be established; that is, the desired stretches of DNA coding for known things could be inserted at will into the human genome. As I recall, only two constructive ideas came out of this. One was that you would put in a coding sequence for cellulase, which would then enable the individual to eat paper. Rolin Hotchkiss was there, and he liked this particularly. He said this would give him an opportunity to reply to letters: "How much I enjoyed your letter." I will not tell you what the other one was.

Also, finally, I had some erroneous impression of free cocktails, but I will pass that.

TOTTER: My name is John Totter. I am a biochemist lately turned bureaucrat for the second time. I am with the Division of Biology and Medicine at the U.S. Atomic Energy Commission. I suppose the reason for the interest of the Commission in having an observer here is fairly obvious.

FREMONT-SMITH: We have no observers. They are all participants.

TOTTER: I intend to participate. I am looking forward to the meeting with a mixture of interest and trepidation and some skepticism, but with a great deal of hope, also.

GRENELL: Bob Grenell. I am, I suppose, called a neurobiologist. This is an escape term which avoids committing one as to being a neurochemist or a neurophysiologist or anything you can pin down. My primary interest is in the nature of the excitable cell and in whatever relationships one can presume to be functioning between macromolecules inside the cell and the molecular structure of the cell membrane.

Everybody has been talking about his background. I have the unique background for this work of being an incurable romantic and a musician. And I think the only other thing that is necessary to add to this is that I feel some responsibility by way of a small, but very interesting, minor connection with the Office of Biosciences of NASA.

WOESE: My name is Carl Woese. I am a heretical biologist of the Pollard school. My interests center about what is currently called molecular biology, within which discipline I seek out those problems whose solutions are particularly amenable to my own personality, preconceptions, and prejudices. At present I am interested in the genetic code—its underlying mechanisms in the origin of life and in aging. I work for the General Electric Company.

ROBERTS: My name is Dick Roberts, and the address is 5241 Broad Branch Road, Washington, D. C. I am mentioning that because the last communication from your people was a letter saying, "What is your name and address?"

I came because I have found that any conference that Dr. Pollard has anything to do with is very good. Now I am wondering whether I should have come because I never use anything more than arithmetic in any of my biology.

FRIEDENBERG: My name is Bob Friedenberg. I am not sure what I would classify myself as. I work in a group that is unique, associated with Professor Danielli. It is called a theoretical biology group. I am here to find out something about the field. I am particularly interested in membrane replication.

MANILOFF: My name is Jack Maniloff. I am in the Department of Molecular Biology and Biophysics at Yale and am one of Dr. Morowitz' graduate students. My research has been in dealing over the past year with the rather unique morphology of what Dr. Morowitz has called the smallest living cell. Perhaps I can make some contribution with respect to that. Like Don Hoffman, the other student in our lab, I am here to learn as well.

KERNER: My name is Edward Kerner from the Physics Department of the University of Delaware. I have been interested in biology for a long time, mostly from a very pragmatic point of view. As Dr. Pollard mentioned, there are certain elements of simplicity in what looks like complexity. The same thing has happened in physics many times. In particular, the power of the so-called Gibbs ensemble theory for looking at complicated systems in a simple way has had a certain possible appeal for questions of similar orders of complexity in biological problems. This has been the theme of my interest in biology.

GARFINKEL: My name is David Garfinkel. I am a computer biochemist; that is, a biochemist who has learned programing well enough so that it is not something he does only under compulsion. I seem to spend most of my time building computer models of cellular and enzymatic systems and a little of my time dabbling with models of ecological systems. I should, at this point, caution the ecologists here that I am not really expert in the field. So please do not be afraid of picking up any factual errors I may make.

LEVINS: I am Richard Levins at the University of Puerto Rico, working in population biology, in the coming together of population ecology with population genetics and evolution.

MOROWITZ: I am Harold Morowitz, a microbiophysicist. When I first became interested in theoretical biology, I was a graduate student of Dr. Pollard's and used to try to think of refutations for the outlandish theories he would put forward. My interest in theoretical biology solidified when I was with the Bureau of Standards and we ran out of money to do experiments one year. I am now at Yale with a group that is trying to sort out the very small living cells in the hope that maybe they will be a little simpler and the theory will be a little simpler.

ENGELBERG: I am Joseph Engelberg from the University of Kentucky, Department of Physiology and Biophysics. I entered biology from the engineering and physics side, but have progressively turned away from the physical sciences and become more involved with biological problems.

I have been thinking about why I have been interested in theoretical biology. Probably any person with a theoretical approach believes in two things about the universe. The first, that the universe is basically simple in structure—not trivial, but simple; and the second, that there is a harmony to it—that is, that there are not lots of unrelated things happening but that they are all tied together and can be related to one another.

McMULLEN: I am Arnold I. McMullen from England, and I am now a theoretical guinea pig in one of Dr. Danielli's experiments. I find that I am the first official Professor of Theoretical Biology. It is pretty clear that Professor Danielli does regard it as an experiment because I have the title "Visiting Professor," and he therefore presumably expects either myself or the subject to last for exactly 12 months.

I was quite quietly minding my own business in England as an experimentalist working on the biogenesis of the polyisoprene molecule. I made the mistake of thinking a little too much

about it and putting on paper some of my thoughts, and so here I am, trying to live up to some of the theories which I put forward at that time.

BAUTZ: I am Ekkehard Bautz from the Institute of Microbiology at Rutgers. I am of a mixed background—first chemistry, then genetics, and I am now called a "molecular biologist." I am an experimentalist, and I think I am on vacation now. I hope I will enjoy my vacation.

WATTS-TOBIN: My name is Richard Watts-Tobin, and I am from the Laboratory of Molecular Biology at Cambridge, England. I started off as a sort of theoretical physicist or, really, a theoretical electrochemist. But I was converted to biology by Dr. Crick and was started off on genetic experiments, almost the first experiments I had ever done, which eventually suggested the genetic code as a triplet code. Since then I have reverted a bit, and I am thinking about theory—the theory of electron transfer reactions in biological systems.

ODUM: I am Eugene Odum. I am a naturalist who sees beautiful order in nature, but, frustrated in not being able to understand it simply by describing it, I have gradually become what we now call a "systems" ecologist, or one who studies the relationship between the structure and the function of the ecological system as a whole. To work at this level of organization some team work is necessary, so we have gathered together at the University of Georgia a number of young people who are interested in the experimental approach and the use of such modern tools as radioisotopes as a means of obtaining better information on function at levels above that of the individual organism.

One theoretical aspect that intrigues us is that many of the most basic principles and functions that we all talk about—for example, thermodynamics or metabolism—seem to be the same at the different levels, whether you are speaking of the cell or whether you are speaking of the biosphere. In contrast, the structure at these levels is vastly different; and, therefore, the real difference between levels of organization is in the relation between the basic functions, which may be very similar, and the very different structures through which these functions are carried out. If I have any function here it is to remind the people working at molecular levels that this is not the only level of importance.

For example, if we code in our cellulase enzyme, as was mentioned, we will have to make some other decisions and studies regarding the effect of our new genotype at the ecosystem level, because if we all go out and eat trees, and so on, this will mean a quite different environment! What I am saying is that we cannot stop in our thinking with simply breaking a code in one level. We must always ask the question: What are the consequences of this at other levels, and how will this modify the theoretical picture when the consequences feed back to the original level?

WESLEY: I am Paul Welsey, a theoretical physicist. My interest is in the general thermodynamics of living systems. I have not as yet gone into the subject in any great detail. I wrote up some of my ideas in a paper entitled "Thermodynamic Behavior and Evolution of Living Systems" (not as yet accepted for publication). In other words, I have really not been in this field of theoretical biology long enough to know what it is all about.

YCAS: I am Martynas Ycas from the Upstate Medical Center at Syracuse, New York. I have been working mainly on the analysis of ribonucleic acid; and my main achievement to date is that I have reached a point where, reading the literature in my own field, I can no longer distinguish fact from fiction very clearly.

My reasons for coming here are twofold. I learned that Dr. Slonimski was coming, and I will not have to lead a discussion. My qualifications for theoretical biology are that I reject papers submitted to the Journal of Theoretical Biology.

EDGELL: My name is Marshall Edgell. I am a graduate student at Penn State, working in Dr. Pollard's group. I was trained as a physicist. I am interested in theoretical biology because it takes me so long to do an experiment, that I would like to do it on paper which should be easier and shorter.

FREMONT-SMITH: I am Frank Fremont-Smith. I was trained in neurology, neurophysiology, and did some work on cerebral spinal fluid, although I had not been trained in biochemistry. I then moved into the job of being a philanthropoid. You all know what a philanthropoid is. Fred Keppel was president of the Carnegie Corporation, and this was his appellation for foundation executives. He said, "Pity the poor foundation executive, he needs to be called something." And he said, "I suggest that he be named a 'philanthropoid'." Why? Because he acts like a philanthropist with somebody else's money.

Since I was retired from the Macy Foundation I have been acting, not like a philanthropist, but with somebody else's money, and trying to assist in some way in the problems of communication.

DANIELLI: I am Jim Danielli. I was brought up as a physical chemist. While I was in the process of being brought up, I met a lot of biologists, and I found it impossible to believe that biology was really as complicated as they thought it was. The result was that I moved into that field. I still think I was right, but it is difficult to demonstrate. I am interested in helping to develop a general theory of cells. I must say I am also a very lazy person, so just like you, I prefer to do something theoretically if it can be done.

QUIMBY: I am Freeman Quimby, trained as a physiologist, plant morphologist, and microbiologist. I came to NASA in May 1960, interested very much in a subject that Joshua Lederberg called "exobiology," which is life, if any, beyond the exosphere of the planet Earth. I came to this meeting because I have been moved for some time by the kind of argument that Dr. Pollard gave us when he spoke a few moments ago. I do not know whether he is really aware of another fact or not, but the contemporary scene in science has been marked by the movement of physicists into the field of biological science. These are good physicists, they are not regarded as heretics by their colleagues, and there is a long list of them. They are not all here, but some of them are. Since I am not a physicist, I came here to listen and to learn.

KLEIN: I am Chuck Klein. I am a microbiologist and have been for a few years. As a matter of fact, I took my first microbiology course from Dr. Starr down here at the end of this table. I have been interested for a long time in the subject of structure and function and have done some work on yeast mitochondria. I am very much interested in the origin of these and other organelles.

I have a sort of personal professional interest in being here. About 9 months ago I was persuaded to join the space program (I think by some of my worst enemies), and I am now with NASA at the Ames Research Center, where we have set up a rather large life-science laboratory. Among the types of work going on there are experiments on chemical evolution and on proteins, or proteinoids, of cellular organelles. Thus, I have a dual purpose in being here: I am spying for the rest of my outfit, and I am also here to learn and contribute on my own.

FORRO: My name is Fred Forro. I am sort of a mixed character. I started out as an M.D., but somehow I did not see enough science in that. Then I found an individual named Dr. Pollard who had turned from physics toward biology, and I found this very encouraging. Now I have the title of biophysicist in the Department of Molecular Biology and Biophysics at Yale. I am listed here as a discussion leader. I accepted this with some trepidation because I cannot

imagine a worse choice for a discussion leader, especially as outlined by Dr. Fremont-Smith. But possibly there will be enough discussion to help me out so that it will not go so badly.

The major reason I am interested in this conference is not so much because of theoretical biology but rather because I have always been intrigued with the idea of de novo cell synthesis. I broke away from medicine because I thought the most important thing about the living process was the understanding of the cell, and my idea of what that means is someday to reconstruct it. I have been diverted from doing anything about this because most people do end up analyzing cells that exist. I ended up looking at DNA, the DNA of bacterial cells, from the point of view of how this replicates. It is kind of refreshing to come back to something which has this title and to start to think about it again, and I hope I become catalyzed.

STARR: My name is Mort Starr. I am a bacteriologist. I earn my living as a teacher of ecology and, unfashionably, as a taxonomist. I am probably here because I am essentially a militant antitheorist. I am a whole-organism biologist. You will be hearing from me on that subject.

DANIELLI: Thank you all very much. Dr. Fremont-Smith, you had some disidentification to make?

FREMONT-SMITH: The reason I want to speak at this point is that it seems to me that what has happened as the result of this self-introduction is that we have all become more aware of the extraordinary resources that we have in this room. We have not become nearly as aware as the resources are, actually, because each one of you has only touched very lightly on one or two of your resources, and you all have many, many more. Of course, one of our functions in the conference is to explore and share each other's resources.

I like to say this because I really think it is quite an extraordinary thing—the capacity, the functional mental capacity, background and experience, of a great variety of sorts that we have here. Our job is, I hope, to utilize as fully as possible, as pertinently as possible, and sometimes impertinently, this enormous resource and, also, to build liaison—lines of friendship and of common denominators—among the group so that the combined resources, or the combination of resources, will go on for many years in various ways.

One of the great advantages is that a lot of you have not met previously. This will no longer be true. Interactions can start which will, and may, go on more effectively because you will have shared with one another more than is possible if you were making just a series of speeches. Thank you.

GRENELL: I was just noticing something as a result of everything that had been said here, and I wonder whether this has any bearing on the meaning of theoretical biology that we are going to be discussing. I just made some notes as people were speaking here, and this looks like a very subversive group. It consists of numerous ex-physicists; Freeman Quimby is an ex-biologist; Dr. Forro is an ex-M.D.; and there are a number of molecular biologists. By this term I assume they mean very small biologists. So I begin to wonder if this has any bearing on the nature of the field.

DANIELLI: You think we are a lot of unfrocked priests? I think every new doctrine is initially heretical.

REYNOLDS: I have one additional comment to make. I noticed that there seemed to be a lot of people from NASA at this meeting, and I propose to reduce the number by 20 percent. I regret to say that I can be here only this evening. I must leave tomorrow, so I want to express my regret that I will not be here for the rest of the conference.

DANIELLI: In that case, you may be the only member of this group who will read the report when it comes out.

HOFFMAN: I have a question about this format. Do these meetings follow each other, A precedes B and so forth, or are they concurrent?

DANIELLI: No, they follow one another, except insofar as they are concurrent.

FREMONT-SMITH: This is a perfect statement to go with this meeting. This is exactly the way it should be. It is order in disorder, when disorder is needed in order to get order.

PART I. ON DE NOVO CELL SYNTHESIS

CHAIRMEN: E. C. POLLARD and J. F. DANIELLI

A. MACROMOLECULAR ASPECTS

Discussion leader: E. C. Pollard

POLLARD: I am very happy to see some new arrivals. I see that Dr. Szent-Gyorgyi has arrived. We are delighted to have him here; just to look at him is going to do me good while I am chairman of this session.

I would like to say just a few words about one purpose of this conference, which is secondary to the actual conference itself. I hope that all of you will attend the business meeting—and please do not be put off by this—the purpose of which is to try to find more wisdom to guide NASA and AEC in the setting up of theoretical biology and supporting it.

I would also like to give you just a little more background than Dr. Reynolds gave you last night. Over the past 18 months or so a very small committee has been meeting; and this very small committee has consisted, besides those in granting agencies, of Dr. Morowitz, Dr. Danielli, Dr. Engelberg, Dr. Henry Quastler, and I. This conference comes out of the deliberations of that group, and it was always the intent that there should be a wider number of people who would advise as to the future aspects of this subject of theoretical biology. So, when we do meet for the business meeting tonight, please bear this in mind: We are simply trying to find out what you think and to put these thoughts together in some way so that support can be given to this subject if it should be given.

Before I go on, I mentioned Dr. Henry Quastler a moment ago. I would like to say a couple of words about Dr. Quastler, and I would very much like to have these in the record. I miss him at this session like an arm. Henry Quastler was a really remarkable person. He would have been wonderful to have had here. He had the kind of interest in the stimulus of new ideas, and his response to the challenge was such, that it did not show in himself vividly but it was very easily communicated to others. In fact, in this respect, a quiet, gentle and anything but strong outward-going personality had the most extraordinarily powerful influence on other people; and this is, in itself, of interest.

I would like to say another thing. Like Dr. Quastler, I am an American citizen by choice. I think it is something we should say to our credit as Americans that a man like Henry Quastler, who had this keen intellect and essentially lived in a world of intellectual achievement that was his whole and entire life, could come here and be in our culture. I think he flourished in it. I really think that the national environment of the United States provided him with a greater satisfaction than any he had encountered before. I really believe he was happy. And I believe this is to our credit that a person such as Henry Quastler could come here and so quickly find himself and find contentment here.

I do not want to burden an already burdened conference with such words, but I would at least like to put these remarks on the record.

It falls to my lot to start the Conference on Theoretical Biology, and I do this with a mixture of pleasure and fear. I find the subject extremely interesting. I find it challenging. I find on the one hand that it is a relaxing thing. I look forward to letting my hair down very soon, and I look forward to a lot of interesting discussion back and forth. At the same time, I find it very difficult. On the one hand, I think that anything one thinks about that is concerned with the intimate detail of living things is absorbing, interesting and exciting; and one could go on with it all day. On the other hand, I am also aware of the fact that one has to superimpose his conditioning and his sort of mental prejudices (and these include, of course,

the background he has in physics, or in biology, or in chemistry) on the actual operation of a living cell. A living cell is subtle; it is simple; it is rational; and, above all, it is itself. It does not seem to matter what one's conditioning is, the cell will require in him an understanding that he meet it on its own terms. So the thing which is difficult is to stand up and start to discuss the theory of a living cell, which is not simple.

I would like to say a word or two about what I conceive theoretical biology to be, or what I think its function should be. In doing this, I would like to go back to physical science for a little while and just draw an analogy for a moment. If one thinks of the very exciting time in physical science between about 1896 and 1910, we have a bit of an analogy for the situation that we have at present.

In 1896, or approximately then, the electron was discovered, radioactivity was discovered, and X-rays were discovered. In the years that led up to 1910, all of these things were exploited and developed in an amazing series of experimental researches, so that by 1910 one might honestly say that X-rays had become commonplace, radioactivity had become commonplace, and electrons had become commonplace.

In this era, the essential method of work, even in physical sciences, was what might be called intelligent experimentation. By the end of 1910, just after this time, the experiments of Rutherford began on the structure of the atom. He showed, by what amounts to intelligent experimentation, that the atom, in spite of all the difficulties with it, must have a nucleus and that the electron must be fitted in the atom a certain way. By 1912 a sudden change came, because in 1912 it was realized that we would not understand the atom in terms of intelligent experimentation. We would have to understand the atom conceptually and differently in concept. We would have to actually forgo the notion that we could think of the steady and uniform absorption of energy by an atom. Instead, we had to make rules for whether it could be absorbed or not; and when we did make these rules, then the picture of the atom emerged.

Now, the whole of that part of the knowledge of the atom—all of the interpretation of spectroscopy and all of that—really is wholly theoretical. It would not be correct to say that anywhere in that aspect which followed from the Bohr theory did any of our conceptualization of the atom lie in anything other than a theoretical interpretation.

The whole question which really lies in front of us at this conference is this: Has biology—which has certainly made equally astounding advances, and to my mind more astounding advances, in the last 15 years than were made in the previous 15 years I just quoted—also come to the stage where the next understanding of living processes will have to be conceptualized in terms of theory? My feeling is that it has come to that stage. One of the reasons why I am so wholeheartedly willing to devote my time outside the laboratory and to this conference, is the belief that, indeed, this is so. That belief does not get anywhere; and, in fact, no subject, I think, is more devoid of basis than theory if that basis is not related to some achievement of theory. A theory which is just a philosophical theory (and I do not use that word "just" intending to be contemptuous although I cannot help feeling that I mean it a little that way)—that is, the very broad type of theory which is very pervasive and very full of faith—is really, perhaps, not what we are after. What we are after is a theory that takes detail and moves this detail into the sort of realm of confident interpretation and gives a basis for extending this confident interpretation into realms that we did not think about at all before, just as the interpretation of the hydrogen atom by Bohr led to all the succession of additional things that were not thought of at all and culminated in an all-inclusive theory, which really encompasses the whole of chemistry and the whole of physics, at least as far as atoms are concerned.

With that in mind, one reason for the titles that we are discussing in this conference should be explained. In planning a conference like this, we had to look at areas where it seemed probable that the contribution of theory to the areas of knowledge we are discussing

would be great. We had to pick, and so we picked, rather sharply, something which many people are looking on a little vaguely. We picked rather sharply the idea of what it would take to synthesize a cell de novo—in other words, conceptually to make, stage by stage, all of the elements from the carbon, nitrogen, oxygen, phosphorus, sulphur and hydrogen which form the cell—conceptually to make these step by step, so that at each point we are confident that we can do it and, finally, to end up with a system which actually is a living cell, which has those attributes of life to the extent that all would agree that the cell is a living cell.

What will it take to do this? It may be that all it will take, as Dr. Roberts says, is to isolate three polymerases—the polymerase for DNA, the polymerase for RNA, and the polymerase for making the peptide bond—and put these together in a suitable container with the right parts and say "boomph" and you will have it.

This is possible. The question is: While this is possible, would one ask for a grant to do that? I think one would not ask for a grant to do that with any expectation that it would be supported unless one also gave some basis for believing that it would work—and here we start. The basis for believing that it will work will involve making a conceptual picture in our mind of the reaction vessel, the nature of these polymerases, how they work, what will actually result from them, how they will interact, and whether, in the long run, the necessary organization which is present in the cell, which we are going to look for and will all be willing to agree is a cell, will actually emerge from this system. When we think about it this way we think that this aspect of our grant application is not going to be done in the laboratory; it is going to be done in the head. The question immediately starts to appear sharply: Is there not really something theoretical that we should start to say about this process of putting the three enzymes together in parts to see whether, first, they could work and, secondly, if they could work, how it would be wise to put them together so that they would have the maximum chance of showing that they worked?

I would like to say that I believe that there are two very clear areas in which we cannot possibly understand the living cell without theory, and I would like to state these very briefly and shortly. Then I would like to start to take something more substantial that you can get a handle on.

These two areas are, first, what I will call the fine structure, or the ultrastructure, of the cell. I do not believe we are going to be able to take a mural of the size of the wall here (something we are starting to do at Penn State incidentally), and actually draw a section of the cell in such a way that we believe it could be right without a considerable consultation of theory. I do not think we are going to be able to take electron micrographs. Even if we take electron micrographs or field ion-emission micrographs or whatever these are and simply, so to speak, project them on the wall, even if we use electron micrographs and even if this aid to the eye is possible, it will take, as an electron microscopist full well knows, a theory to interpret the electron micrographs as he sees them, to make the pattern that is actually there.

I am very impressed by the fact that even such beautiful things as mitochondria will not permit this. It would almost seem as though mitochondria and viruses were designed for electron microscopy because they show up so well. But, even so, I have noticed that the conceptual pictures that are drawn are, nevertheless, derived pictures. They are not pictures that one can hand to a graduate student and say, "Look at them! Here they are."

This is one area. The other area in which I feel confident that theory will be necessary is in the area of understanding the mechanism by which the cell actually does its job. I do not think that we are going to be able to say how DNA is synthesized, how a protein is synthesized, how a membrane works, how a permease actually operates, in the true sense of the word "how" without theoretical understanding. I do not think I can conceive of any method by which one can actually continue, so to speak, to tease apart the cell with micromanipulators, poking

and poking and looking with the eye the whole time, and come up with a mechanism by which a system of enzymes follows a pathway and forces operations down the pathway. I do not see how this can be done.

These two areas then, of the intimate structure of the cell and of the precise function of the cell, are the two areas where I feel confident we must have theory to guide us. What I would like to do, rather than talking in this philosophical vein, is to take two aspects which I have spent a little time worrying about and fill in quickly the kind of reasoning that leads one to believe that one will have to have a theoretical basis for the nature of the operation of the cell. Then I would like to start in a little while to sketch the way the living cell, say a bacterial cell, actually looks.

The first thing I would like to talk about is a very, very simple consideration of the rates of synthetic processes, or any process which can occur in a cell. Let me illustrate this by something which is quite new and which I have done in a very sketchy way in the last 10 days or so. It concerns the mode of operation of an enzyme, beta galactosidase, which is one of the enzymes I know a little about.

Recently it has been realized that in a bacterial cell there is approximately one of these enzyme molecules per nucleus, and so we have an interesting situation. If we are prepared to know the number of cells we have in a culture and if we open these cells by some fairly rough treatment, we actually have an idea of how many molecules of this enzyme we have. Furthermore, in working with this in the situation where we have managed to open the cell, while we may have damaged the cell badly, we are still looking at an enzyme which is somewhere close to its actual state in the cell. This is opposed to taking, for example, crystalline catalase and looking at it in a pure water solution. We are looking at this thing somewhat as though it was in the milieu in which it actually works.

To make this story very short, because this is not intended to be a place where one reports results and data and things like that, I can say briefly that if we take a culture of cells, smash them open and give them a substrate to work on, in 1 second we actually have 4×10^3 substrate molecules per second actually processed by this enzyme. In other words, the enzyme works at this rate in the measured system.

It is difficult to calculate theoretically what it ought to do, and this is something which I have been trying to bring down to a better basis by doing background experimentation. The sort of theory which you can work on is either clumsy but correct or elegant but just a little distorted.

If we take the clumsy but correct theory, then what we have to say is this: For the collision process the enzyme is represented by a little region where it works, shown at A in figure 1. This is the small unit which takes the double sugar and splits it into two single ones. We start the double sugar moving around in a solution, as shown; and it goes into motion, as indicated, until ultimately it finds the site on which it occurs. The average time it takes to go from start to A will be concerned with the collision rate of this metabolite with the enzyme. The probability W(r) of reaching the critical volume in a time t from a distance r is:

$$W(r) = \left[\frac{1}{(4\pi Dt)^{3/2}}\right] e^{-(r^2/4Dt)}$$

where D is the diffusion constant in cm^2/sec (ref. 1).

This is a nice method of working, but unfortunately, as anyone who plays with this knows, it lands you in very great difficulty. Long ago, for purposes of collisions in colloids and in other areas, this was abandoned, and a substitution was made in which, instead of thinking of the enzyme as looking like that, a little spherical bulge was imagined on a surface, and this is the collision point (fig. 2). This is endowed with a radius. The impinging molecules are

treated as points. Their radius is joined in with the radius of the "bulge." Now we consider what happens as a diffusion process into a hemispherical region. The process of diffusion and the process of Brownian motion are actually equivalent; and the two equations do, essentially, give you the same result. The only difficulty is, you see, I have changed the picture a little bit by the introduction of the radius.

For this calculation I must also assume that, when a collision occurs at a point, I effectively remove the metabolite from the solution.

With this approximation, one can say that there is a concentration C_∞ at infinity, a long way away from the surface and the collision rate ϕ turns out to be the following: $\phi = 2\pi DRC_\infty$ where D is the diffusion coefficient. If one substitutes in the numbers for the beta galactosidase substrate (lactose), this is used at 0.01 molar (so we know C_∞), and we can use a reasonable estimate of this radius (10^{-7} cm). The diffusion constant for sucrose in this sort of medium has actually been measured and is known to be approximately 10^{-6} per second, and it turns out that the number which should be found is 3.7×10^6 per second. Therefore, one thing is clear: the enzyme is not functioning in terms of collision. This is fairly well known; but what is interesting is that a ratio of approximately a factor of 1000 occurs between these two. Thus the actual processing of this substrate and the probable rate of collision are different by a factor of 1000.

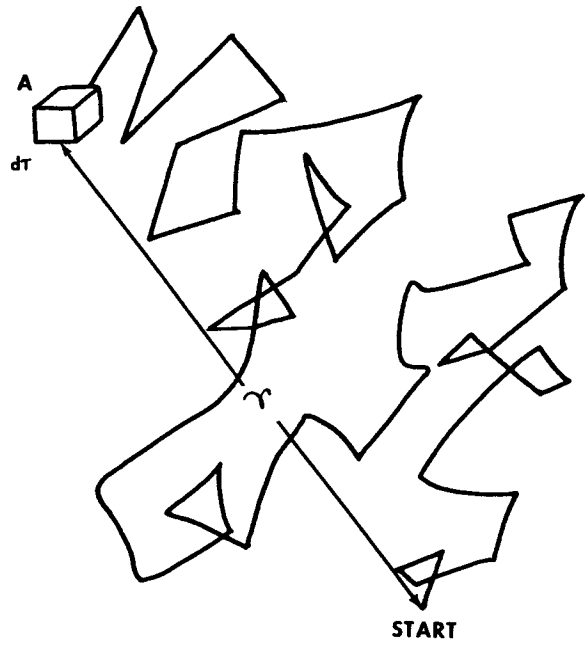

Figure 1.—Indication of the Brownian motion of a metabolite as it traverses the distance to an enzymatic site at A. The volume of the critical region of the site is $d\tau$.

This is not hard to understand in several ways. Incidentally, I think I would like to suggest a very simple theoretical problem for those of you who know statistical thermodynamics: If we have a region of volume V and we have on its surface some element which requires an energy E to force some operation to occur, what is the length of time which it would take by fluctuations—fluctuations of the type we find in critical opalescence, for example—for this energy to arrive randomly in a concentrated fashion? If this is worked out anywhere and applied biologically, I have not found it. I would very much like someone in the room who does know this to interrupt now, or he can tell me at lunchtime. The reason behind the operation of an enzyme might simply be that there is a critical surface site on which something arrives by collision and then, by waiting for a length of time which corresponds to the difference in the two rates given in the last paragraph (which can be estimated as something like a millisecond), the energy concentrates and off goes the substrate. In any event, the evidence is that enzyme rates are slower than expected from collisions and that this diminished rate is somehow concerned with an activation energy.

This consideration of the enzyme process now forces us to look at a chain of 10 synthetic steps. The biochemists are very fond of taking a substance like glucose and showing that it follows a metabolic pathway, and these pathways are nearly always somewhere in the neighborhood of 10 enzyme steps. So now we have to ask ourselves what would happen if all the

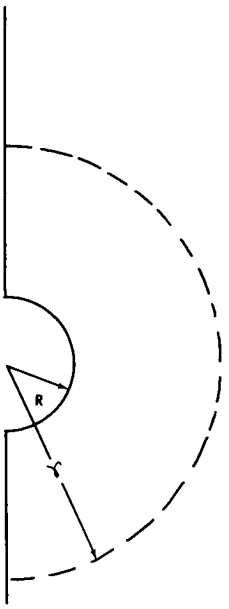

Figure 2.—Replacement of three-dimensional Brownian movement by diffusion to a capturing radius R.

elements of this pathway were actually separated, segregated all the way through the cell, and it was necessary for item 1 to be made, to build up to a concentration sufficient to make item 2 work and item 3 work, and so on, until, finally, we came out on the far side? Even if all we had was a collision frequency and no more than that—in other words, if all the reactions operated purely as a matter of collision frequency and nothing else—it would still be very difficult to see how all these things could process themselves as quickly as they do. But when we have (1) the fact that the reaction is 1000 times too slow and (2) the necessity that there will have to be energy provided at each reaction, we are really led to invoke a system like figure 3, in which we have a set of enzymes and, presumably, another set nearby. Now we have to consider the correct entry into one end of a metabolite which, we will say, has to be glucose and not, for example, hypoxanthine; and the substrate must somehow be processed. Furthermore, we must not have to wait for the energy to accumulate in one place after it has once accumulated nearby, so that there is also an energy transfer process needed for the completion of the enzymatic pathway.

If that is not a system for theoretical consideration, I have never seen one. This type of picture is necessary to discuss; and here we call for Dr. Szent-Gyorgyi's submolecular biophysics, which corresponds to the description of the operations within the molecules of biological systems. We need it, and we need it sharply. We need it accurately. We probably need to know in quantum-mechanical terms the specific orbitals for things like this.

Let me add one last thing about enzyme systems. These systems abound in mitochondria. Now, it seems (something I did not realize) that mitochondria can replicate, of all things, semiconservatively. This means that mitochondria apparently grow into more mitochondria, so this replication of a cellular organelle is taking place within the cell.

GREEN: That is not generally accepted. That is only one view.

POLLARD: All right then, let me ask, in that case, a question: "How are they made if they are not made that way?"

GREEN: That is a long story, but I think it should be on record that multiplication by growth is one of many possible modes of replication and, to my way of thinking, the least likely one.

POLLARD: Fine. In some respects that makes me feel happier still, because I must admit this mode of replication bothers me a little bit. I have difficulty with it, but what I do not have difficulty with is believing that, no matter how mitochondria are made, they probably have to be made in some sort of packets. So there must be a sort of fundamental unit in mitochondria which is almost precisely this strip of enzymes which I am talking about and which, for quite different reasons, is something I seem to want to have.

GREEN: Yes, that I certainly go along with.

POLLARD: So, all I am saying is that there are two quite different attitudes that would lead you to think this way. Actually, there is a third avenue. This is an old radiation experiment which we made at Yale some years ago that showed that if one destroyed (by radiation) the ability of glucose to be assimilated by the cell, the radiation target would seem to correspond to a packet about this size.

GREEN: There is a body of evidence now which suggests that a number of the glycolytic systems of enzymes are actually organized in the form of membrane-bound structures* where the enzymes are, in fact, arranged as you have indicated and predicted correctly—that now, experimentally, it is possible to demonstrate that such is the case, at least in the membrane of the red blood corpuscle.

POLLARD: Another thing I would like to discuss is in this same general vein. This concerns the synthesis of DNA and protein, and let us consider DNA first. If you take the fastest rate of synthesis of a DNA bacterial virus and if you assume (well, we know now that DNA is in one unit) that the DNA is made in one place, and this is an essential assumption of this calculation, then 4000 bases are made per second.

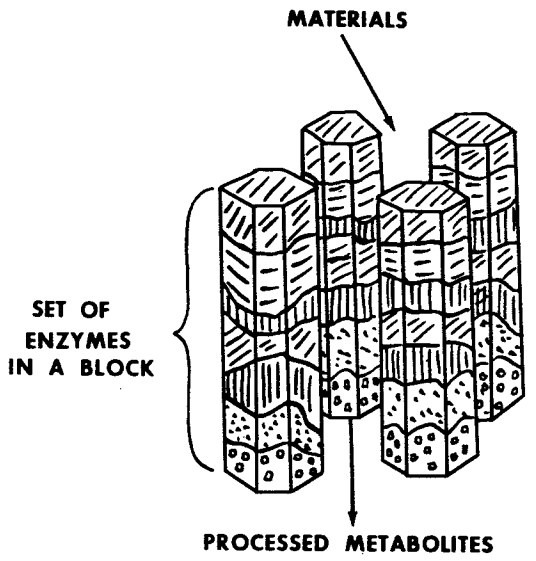

Figure 3.—Schematic representation of a set of enzymes in a block, capable of acting efficiently on material entering the cell and of passing energy efficiently through the solid state from one enzyme to the next, as needed.

These 4000 bases per second are made really very accurately. They are put in place accurately. Incidentally, when you start to look at biology theoretically, right away you begin to see some new experiments or, rather, old experiments for which you need new data. The whole story of how many mistakes are made, at what rate of replication, is something which you cannot find easily in the literature. I do not know anybody who has studied the formation of, for example, phage, at different temperatures to see what the mutation rate is of all classes of mutation as a function of the measured rate of synthesis of the DNA. Moreover, I have not seen it compared with any attempt made to modify the number of these bases. This class of experiment appears interesting to a theorist. It does not necessarily appear interesting to a person who is just a phageologist.

Consider these 4000 bases per second. Again use this calculation where I say that the rate of formation ϕ is

$$\phi = 2\pi DRC_{\infty}$$

Putting in the known value of ϕ and then putting in reasonable figures for the diffusion constant and reasonable figures for the radius and calculating out the concentration to infinity, it works out to be something in the neighborhood of 4×10^4 free bases per cell. This represents, of course, a relatively small fraction of the total bases present in the cell.

This calculation is based on the idea that every collision works. Every collision, in this instance, is really a little subtle because we have to take a base, sugar-triphosphate, which must find the proper place on the enzyme and must come opposite the proper base on the primer; it must twist and turn its way into the correct orientation, and it must fasten itself into position so that it is correctly placed, and there must be no errors. This calculation of

*B. Salmon, E. Murer, and G. Brierley, unpublished studies.
H. Hultin, S. Richardson, and D. E. Green, unpublished studies.

the concentration necessary for this process to work is essentially based on the idea that everything sticks and goes as quickly as possible. No possible sort of slowing down process is permitted for this calculation.

I have been making some attempts to measure what the concentration of the bases in the cell is. I must admit that I am an amateur at this. It involves a little biochemistry; and I do not want to say that I have a final figure, but the figure that we seem to come up with is approximately 1000. In other words, there is only one-tenth as much deoxyadenosine triphosphate in the cell we have worked with. This is work by Mr. T. Barone and myself.

I would not like to have my whole scientific reputation depend on this number; nevertheless, I would like to point out that, again, theory suggests to us that this type of number, which is not so laborious to get in the laboratory (it is only laborious for me because I am not a good biochemist, but it would not be laborious for some who can do it better than I can), is of course tremendously suggestive. First of all, it is small—too small for the theory. Thus, either we reject entirely this mechanism of collision—which essentially means we reject what we think chemistry is because we believe chemistry to be concerned with collisions between molecules and rearrangement thereafter—or we say that, in fact, we will have the right concentration of bases, but strongly localized in the cell. Actually, what we find is that this figure per cell is used to give an idea of how small a region is occupied by the bases used in DNA synthesis. Therefore, the number in the cell may actually be in what must perhaps be 1/1000 of its total volume, so one comes up with an idea of a whole organized DNA synthetic mechanism. This is shown in figure 4. The DNA, the two strands of DNA, and the polymerase are shown. This last functions synthetically. In addition, however, somewhere around it is a little region in which we have a concentration of precursors of 2×10^{16}/milliliter. These must presumably be made near it, and so around this polymerase unit is a section of the cell which, in some way, has acquired the machinery for putting the triphosphate onto the sugar and the sugar onto the base. All of this machinery either must be closely around the polymerase or

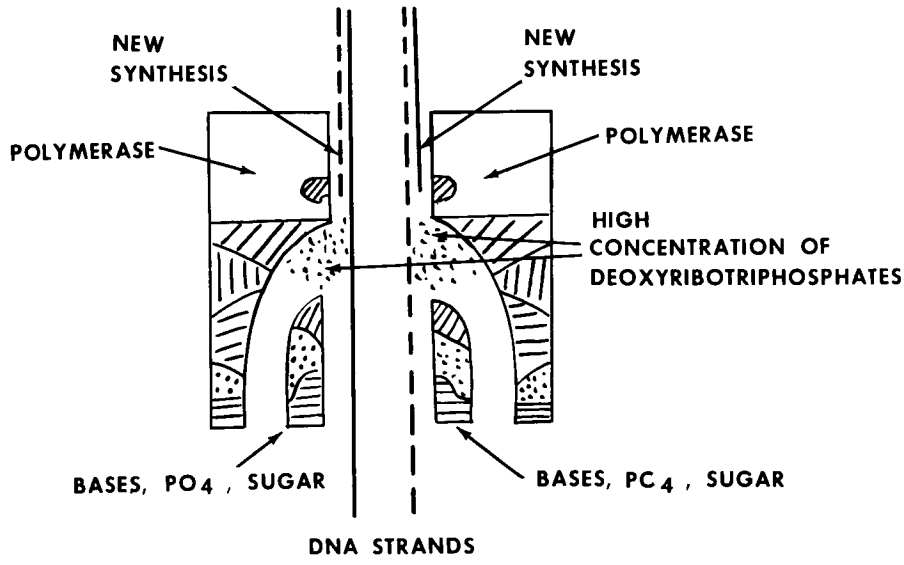

Figure 4. —Representation of the organized set of enzymes near the polymerase. These enzymes make a high concentration of triphosphates in a very small volume right near the point of DNA synthesis.

there must be a mechanism by which a unit made elsewhere is somehow brought down, and brought down quite efficiently, and put into this zone, and then made to work.

Presumably, since the DNA is wound up to be about 800 turns or so of the cell, this is moving around in some manner systematically within the cell or, alternately, the DNA is systematically working its way through the structure.

YCAS: Dr. Pollard, you are assembling this DNA sequentially?

POLLARD: Yes, I am taking the Kornberg method of assembling as right; and I am assuming it goes on each strand from one end and that the primer is a continuous thing and the second strand is made that way.

YCAS: For the entire DNA?

POLLARD: For the entire 130 million molecular weight of DNA.

BAUTZ: There is one problem with your continuity. Theoretically, you need only one polymerase molecule per cell, but there are around 200. If it starts on one end, you would need only one polymerase molecule. I just wanted to say that. It might not have to work all the way.

ROBERTS: That is true if it stays on all the time. It may fall off, though.

BAUTZ: It might start at different points.

POLLARD: It is not too bad. It cannot be less than the sum of the radius of the diffusing molecule and the "little boss."

I think that a factor of 10 is certainly permissible. In fact, let me make it quite clear that I really do not think exact values are important in a certain sense. The thing that is remarkable to me is that my values are the fastest possible; everything else is slower. It is also true that to obtain observed rates there is only one mechanism short of special structure that I can think of. Without special structure, the only way I can obtain these rates is to increase the concentration. Interestingly as soon as the concentration required begins to be greater than that observed throughout the whole cell, both arguments lead you to special structure because you now must put those molecules in in small volume.

MOROWITZ: It is a carryover of macroscopic concepts to a 1-micron box.

POLLARD: You have to say it was essentially distributed uniformly throughout the box or, with maximum probability, in the middle and not try to follow its path around.

DANIELLI: There is still a significant value of R, though. You still, fundamentally, have exactly the same problem. It does not seem to me that this point really matters very much in this system.

Actually, the data that Dr. Pollard counts are really averaged over a long period of time. As far as I can see, it does not matter whether you average over a long period of time to get a result or whether you average over a large volume over a short time, you end up fundamentally with exactly the same conclusion.

YCAS: There is another point, and I would like to return to my original question. This is all very interesting but it depends on certain assumptions which are experimentally testable. I do not think anybody really knows how DNA is made; for example, how many initiation points there are.

POLLARD: I think Dr. Forro does know how DNA is made.

YCAS: I apologize to Dr. Forro, but my point is that this is an experimental question.

POLLARD: I would like to say I do think theory frees one from the bondage of experiment, that is to say, as long as one states his premises. There is no question that many people today are discussing the sequential synthesis of DNA around a circular chromosome from one end to the other, and the evidence is probably in their favor, from all I know.

ATWOOD: It is overwhelming.

FREMONT-SMITH: Temporarily overwhelming.

ATWOOD: Have you seen Cairns' recent pictures?

YCAS: No, I have not.

ATWOOD: You have to see those to realize how overwhelming it is. There is just one growth point.

YCAS: In what?

ATWOOD: E. coli.

FORRO: It depends on the way you accept the data. Cairns (ref. 2) has collected evidence, but I do not believe you can draw the conclusion that this is uniquely true for all the molecules in these cells. He does not get 100-percent yield. He gets very infinitesimal yields per structure. Maybe from these structures you can make such an interpretation.

ATWOOD: The material is very fragile; it is surprising he gets one good picture.

FORRO: I agree, and I think there is evidence that bears out that sequential synthesis does occur in other systems, thus making Cairns' results very probable. The thing I take most exception to is the idea that this is necessarily going to be true for all systems under all conditions, even if we restrict ourselves to bacterial systems. The other systems that I referred to were Nagata's work (ref. 3), in which he looked at the number of prophage per chromosome during synthesis of synchronized chromosomes, and Yoshikawa and Sueoka's (ref. 4), where they cannot pin it to a material structure of linear continuity but they do show a sequential synthesis. However, in each of these cases is a system in which sequential synthesis is not demonstrable. Nagata has run the experiment with an F-minus strain, and it does not behave this way; and Yoshikawa and Sueoka have another strain in which they cannot show this.

I have some long-standing experiments in which I just do not understand if the mode of synthesis is sequential in all organisms; therefore, I would just like to put a reservation into the acceptance that sequential synthesis is always the case. It does seem, though, that the weight of evidence is in favor of sequential synthesis under some circumstances for a large structure; thus, I think that Dr. Pollard's willingness to assume this for purposes of theoretical consideration is reasonable.

YCAS: I would say that the main point is not to be overwhelmed. No single experiment, no matter how plausible, can be overwhelming in the sense that it renders further experiments superfluous.

FREMONT-SMITH: The history of science supports you.

POLLARD: I think this is beginning to be my point about the contribution of theory—that this is only one of many possible things and what we have to do is to ask: Is the experimental evidence good enough to make us think about it? I think we should exert the prerogative of the theorist's being lazy. If the experiment does not bring us good enough data to warrant our using our heads, we should not. In this case I think the experimentalist has brought us enough data so that it begins to look as if we should consider them. All I feel like saying now is that if one takes the sort of normal rates of synthesis and the sort of concentrations necessary for

them to take place, then one is pretty well driven to the idea that the bases necessary for the synthesis of the DNA at one end are going to be specially packaged in some kind of a still (to me) unknown structure. It seems to me that this unknown structure, whatever it is, is something that perhaps will only be discovered and understood by theory.

I, personally, imagine this thing as coming within Dr. Morowitz' 0.1 micron. At the moment very few people will talk about 0.1 micron in a living cell as anything that can be visualized in any way. Therefore, I think it has to be conceptualized; and that is theory, as far as I am concerned.

GRENELL: I think there is a point that comes up here that may sound naive. But if we are talking, from a theoretical point of view, about cells—and everybody has been so far—the question is: Are we talking about all kinds of cells, or specific kinds of cells? If we are talking about things that can be generalized to all cells, then one begins to wonder.

This figure of 2×10^{-12}, for example, is a very small figure which one would think reasonable for certain cells but not as reasonable for others. Thus the question arises as to what are the limitations on the theoretical approach at a particular level that is being discussed.

POLLARD: Again, I am taking the prerogative of a theorist. I do not think it bothered Hans Bethe at all that in calculating the absorption of a fast-charged particle he did it with just pure hydrogen atoms, even though no such absorbing systems are readily found.

Nevertheless, I think we are not making a claim for generalities. The theorist tends to say that if one clearcut, specific, known true case can be analyzed, this is sufficient for his purpose. Whether it will apply generally is a question. In physics and in chemistry in the past, the history is that it does. From the trends we can see in biology, I would say that it probably does. However, I would very much agree that the calculation made for E. coli cells would not apply to, for example, PPLO, which is much smaller. I certainly cannot believe it would apply without modification in the mammalian cell.

GREEN: I would like to point out that, if there is a direction that ultrastructure studies is taking, it is that these smaller components are localized in very, very microareas and spaces between structures, such that it would be possible to obtain very high concentrations, as you predicted.

I think the notion that you are dealing with molecules evenly dispersed throughout a cell will have to be abandoned. The whole problem of achieving the necessary intracellular concentration of substrates and coenzymes is, I think, coming closer now to a solution. There are, in fact, these microspaces; and whatever the laws that govern the behavior of molecules in these microspaces are, they will be the ones that will apply to calculations of the kind you are making.

FREMONT-SMITH: When you use the term "temperature gradients" within these minute spaces, within the cells, this could materially increase the diffusion rate and collision rate. The calculation of temperature gradients gives an enormous increase for very small temperature gradients.

POLLARD: They are almost hopeless. I have tried this. The surface-to-volume ratio is so huge for these small things that the temperature effects equalize very, very quickly; and in a cell of this size, the maintaining of even a 1/10 000 degree difference from one end to the other would be a spectacularly difficult thing to do by ordinary methods. I am quite sure if you put it in a gradient, a Bunsen burner, and heat it over here and have something cold on the other side, you can do it.

FREMONT-SMITH: But there will be continuous heat production at many points.

POLLARD: That is right.

FREMONT-SMITH: And, therefore, there will be continuous temperature gradients from each one of these points.

POLLARD: But the speed of the heat exchange, dQ/dt, is colossal. It is determined by the conduction equation that

$$\frac{dQ}{dt} = \bar{\underline{K}}\, A \frac{d\theta}{dx}$$

where

Q	heat
t	time
$\bar{\underline{K}}$	thermal conductivity
θ	temperature
dQ/dx	temperature gradient
A	area

The area A is so great that no large temperature gradient can be sustained. The transfer of heat is not like that of matter because one concentration is a transport of an actual nucleus that has to move. This is the transport of matter, and the other is the transport of energy of vibration. The transport of vibrational energy can take place without moving a nucleus. I think they are different. Concentration involves actual motion of the unit.

All I want to say is that if you put in the normal constants here and relate them to this problem, as soon as you shrink the size very much this area becomes potent compared to the heat generated, which is very tiny. The actual heat itself is the specific heat times the volume times the density and the volume is so extraordinarily small that the amount of heat is very, very little; thus you quickly see that the temperature gradient is also very little.

I have made the calculation.* At one time I thought an explanation for radiation action was that the molecule would get heated very suddenly by the arrival of the ionization energy within the molecule. I tried to calculate how this would be related to known constants, but I rather quickly gave up because I figured that the time that the heat would remain in the molecule was extremely small.

FREMONT-SMITH: But this would be continuous, would it not?

POLLARD: Yes.

FREMONT-SMITH: Would that not make a difference?

POLLARD: It might.

GARFINKEL: May I cite one example of this which, I am afraid, is also a counterexample to what you said earlier? We have been simulating the glycolysis pathways in the mouse ascites cell

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*If an enzyme is reacting with 1 electron volt of energy 10^3 times per second it generates (or absorbs) 4×10^{-17} calorie per second. Using an assumed thermal conductivity of 4×10^{-4} calorie/square cm/second and a radius of 20 A, we find that dQ/dx = 0.02 degree per cm. In a cell of 1-micron width, this means a very small temperature difference indeed.

and, for the most part, thus far we have been able to assume that these things are in solution as enzymes independent of each other or that you could effectively get a working glycolytic system by isolating the enzymes, pouring them all into the same beaker, and to assume that they do not associate.

There is one exception which is needed to provide a very high local concentration of one particular substrate because the enzyme reacting with it apparently is driving the reaction the wrong way—in a situation not in equilibrium it is moving stuff further away from equilibrium. In this case there is experimental evidence for association of two enzymes, as these were thought to be the same enzyme when first discovered, and it was quite a while before they were resolved (ref. 5).

POLLARD: That is very interesting, because I must admit that when I tried this collision, kinetics to the glucose metabolism of cells, I found I came out with fairly low numbers being required. The concentrations required were quite low, and this would agree with your findings. Oddly enough, the ones that seemed to require the high and strange concentrations are these synthetic things where you are more sure that they take place at one point. They also take place with this great speed. I agree that it would not be necessary for intermediate metabolism to invoke anything structurally special, as far as we know.

May I ask, have you put in the known rates for the separate enzymes?

GARFINKEL: As far as they are available, yes.

POLLARD: And the rates can be sufficient if one enzyme is at one point in a cell and another one far away?

GARFINKEL: That is right, with that one exception.

TOTTER: Does the concentration that you have to use for the steady state agree with the osmotic requirements of the cell?

GARFINKEL: That I do not know, but these enzymes and their substrates would form a sufficiently small part of what is inside the cell osmotically.

TOTTER: Not enough to cause trouble.

GARFINKEL: That is right.

POLLARD: I will not be much longer on this theme. My feeling is that what I am putting forward has a certain degree of emotional conviction. The next thing to do is to perform an experiment. The emotional conviction I have is that the synthetic mechanism for DNA is more than just the polymerase and that there must also be a space around the polymerase in which one can find the four bases and, also, in which one can find the remainder of the mechanism.

I am not a person with a good sense of space relations, but it seems to me that we do know this structure of DNA reasonably well and we know it is coiling. It is coiled in two ways, at least a minimum of two ways: it is coiled in the Watson-Crick manner, and it also has to fit into the cell. It is 800 times longer than the cell, so it has to coil into the cell somehow; we know that.

It is coiled in this double way, and it either has to move through while uncoiling—through this polymerase mechanism which somehow provides the basis for it—and so either one or the other must move along. It seems to me that we already have a pattern which a person with any kind of a three-dimensional mind and any kind of sense for what goes on could set up as a theoretical project. Now we can draw up a list of all the items, that I can think of as theoretical projects. One of the by-products, or maybe the major product of this conference, should be that.

THEORETICAL PROJECTS

Examine and characterize as many purely physical processes as possible. Write out the conclusions clearly so that they can be understood.

DNA

1. DNA rotation.
2. Topology of DNA replication.
3. Genetic "hot spots" and physical coiling.
4. Separation of DNA at "readout."
5. DNA elasticity.
6. Precision of synthesis.

TRANSFER

1. Transport of protein to organelle regions.
2. Assembly of mitochondria.
3. Energy transfer in enzyme "blocks" (multiple enzyme systems).
4. Metabolite transfer in channels.

STRUCTURE

1. Packing of protein and lipid.
2. Contractility and "transferases" (in transport).
3. Theory of multiple chains for genetic complementation.
4. General theory of protein secondary and tertiary structure.

To go back to what I threatened to start off with, to give a picture of the cell and then suggest some things that I can also see are necessary, I would like to suggest a second one—namely, the precision of synthesis. I suspect one can actually examine the precision of synthesis for DNA, RNA, and for protein.

To come back to what I was going to say: what begins to appear for the picture of a cell, and which now starts to invite theoretical discussion, are such things as these. First of all, this cell is drawn on a very much larger scale than normal; we have two layers. In this case we have the outer layer of some kind of polysaccharide structure. Incidentally, this structure is beginning to become fairly clear; it can be drawn. I do not know how to draw it, but it can be done. The attempt is in figure 5.

And then we have Dr. Danielli's double layer, which is the membrane. Inside this we have, presumably, some kind of a system of these blocks of enzymes, which are present and which are channeled in some manner. The surface of the cell is drawn in this sort of way and into the inside region we are not permitted just to quietly draw something repetitive because we are almost certain they are all different. They may, perhaps, (and this is possible) be patches, but it is doubtful if they are.

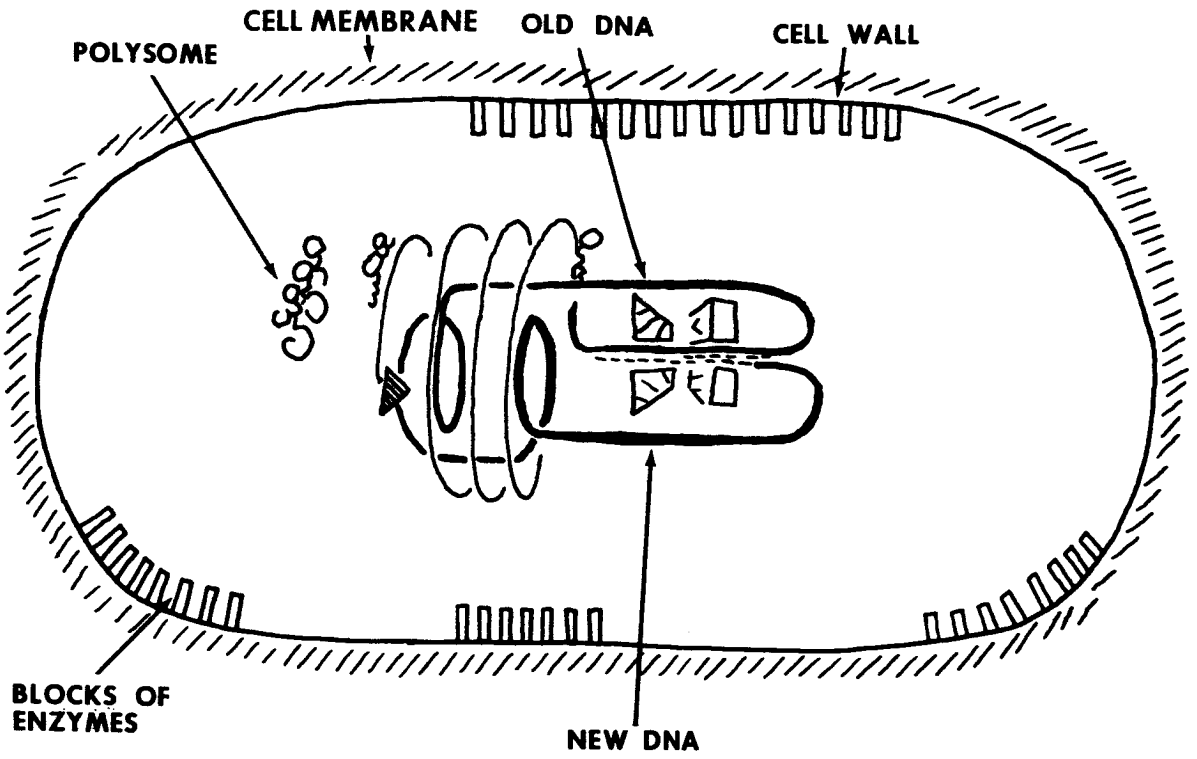
We have, presumably, some sort of a gateway mechanism, and these are the "permeases," if you like that word.

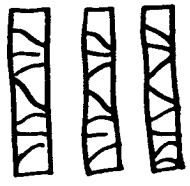
GREEN: Dreadful.

POLLARD: Is "pumps" all right?

DANIELLI: Stick to permeases.

POLLARD: These are the permease molecules which can somehow pick here a glucose and here some amino acid, or something like that, and which can put them into the proper pathway and reject the others so that the space does not get jammed. Somehow or other we have to see





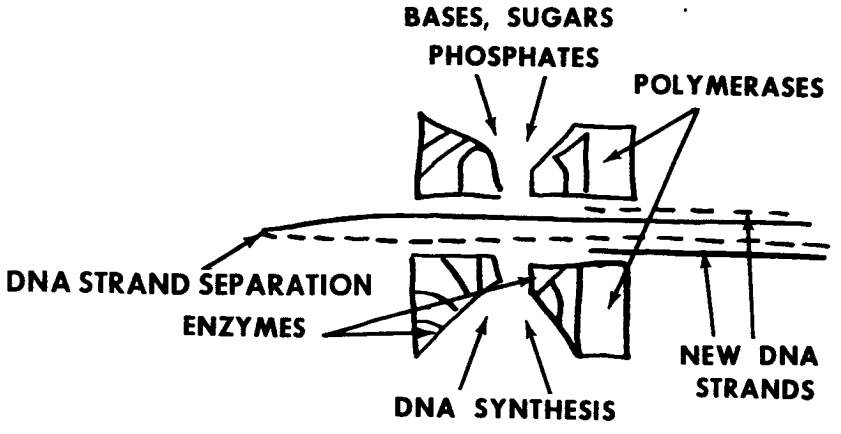


Figure 5.—Diagram of a cell.

this extraordinary mechanism—look at this in a sort of sectionalized way, in terms of DNA. In DNA these turns come around. If this is how it is done (this is what Dr. Forro is going to tell us about; I am setting up his part of the conference ahead of time), there are sectional turns of this DNA going around. Somewhere at some point (and this is a sort of once in a while) will be this factory which contains these triphosphates; they are not going to get there by a miracle so, presumably, there is a relationship to these mechanisms which are forming things inside the cell.

Now, there is much more than that. What I have drawn is essentially the outward apparel of the cell. In a sense, this is dead and gone. The life of the cell, the whole thing which is the vivid, actual living process in the cell, is down below. We are going to be impelled to think about this because what is supposed to occur is that, coming off these strands (DNA separation) at some stage (this DNA is supposed to separate here), we see this momentarily like this. We will pull the message. So, looking again always in sections, we pull the message off. This message is then, in turn, conveyed by some mechanism to five or six objects. This is somehow strung along and on the far side one unit comes off of one of these proteins. The proteins are made in blocks, and somehow one of these units of protein gets into its structure.

When we make a picture like this, we realize that it is a far cry from the crude reaction vessel idea. I will put a little thymine out here, and this will then be doing what I want it to do here. The mechanism by which the thymine gets inside, gets hooked onto all the things it has to be hooked onto, and finds itself in the synthetic region for DNA is a long and multiple mechanism.

What I would like to suggest is that we have still more theory to think about. There is the energy transfer in enzyme blocks. There is the metabolite transfer in channels. Another one is the separation of DNA and "readout." This is where the messenger is actually taken off. Therefore we have sets of problems (see "Theoretical Projects" above) that come to us. I would like to suggest another one, and that is the transport of protein to the organelle regions, the regions where the enzyme works in blocks. If they do not work the way Dr. Garfinkel says they do, then they have to be put in an organized form. But we know that they are made somewhere down in this secondary structure, the structure of the DNA and RNA which is secondary to the factory.

DANIELLI: Are you postulating that the sets of enzymes involved in one organelle are made sequentially along a DNA strand, or are you postulating that they are made in different chromosomes and then are assembled by some special mechanism?

POLLARD: According to Demerec, the cistrons that make these proteins are put together so that they are probably made sequentially; thus you can imagine this whole mechanism of production of one of these blocks of enzymes being read out on the DNA.

BAUTZ: Not necessarily though. The genes of the arginine pathway are distributed all over the genome.

POLLARD: Nevertheless, there are pathways for which the genes are contiguous.

BAUTZ: This is right in most cases, but there are exceptions.

ATWOOD: The reason they are contiguous in such cases may be irrelevant to the problem of organelle-bound enzymes, which is that they can all be controlled by a single controlling element that turns them on and off at once.

DANIELLI: You do not mean turn on and off; you mean made or not made simultaneously.

ATWOOD: Yes, I mean that the members of such gene ensembles are linked so that the enzymes will be synthesized in proportional amounts.

YCAS: This is an important point. Enzymes controlled by the same operon are not synthesized in equimolar amounts.

ATWOOD: They are proportional.

YCAS: They are proportional, but the proportion can be varied, for example, by changing the temperature.

DANIELLI: The point is, however, is it a special case when there is an operon for a particular system—that is, a special case which has been overinvestigated compared with other cases because it is easy to handle—or is it really representative? Even if related enzymes are made by peeling mRNA continuously along a strand of DNA, there is still a problem of arranging the resultant enzymes to be put in a packet. But if the mRNA's involved are made on different chromosomes, then a still more difficult problem is involved.

POLLARD: That is right. If they are not made contiguously, then this transport problem becomes a serious one and one which is of great interest. How do these things know to marry up and form the right line? It sounds like a miracle to me.

Just offhand, I remind you that these form fast. One must remember that the structure I have drawn (fig. 5) is all made and finished and in beautiful shape in 22 minutes.

DANIELLI: Your postulate would mean that the messengers come off particular sections of DNA and get packaged together again in another part of the cell where the protein synthetic mechanism is. That could, I suppose, conceivably cover both cases, could it not? That is, where you are dealing with one operon and where you are dealing with messengers from different chromosomes—they could still be packaged in one place for protein synthesis.

POLLARD: To tell you the truth, I have run out of my individual ability to think. All I am suggesting is that this should be thought about. I, myself, have not reached the stage of trying to imagine how a system of 10 enzymes can become assembled, put onto the membrane (or close to the membrane), and put in their functional place. The strange thing that begins to form in my mind is this peculiar, weird biological crystallization process which seems to occur—this sudden requirement of the ordered form in a complex system. Remember that these are different enzyme systems forming. "How can that happen?" is the question I am asking. Really, all of these things come down to that question.

ROBERTS: What is the matter with the concept of a jigsaw puzzle to account for the assembling of these systems? They more or less shake around until they assemble as a jigsaw puzzle might.

POLLARD: That is right, but I think that can be calculated theoretically. I am not saying I can calculate it, but I am sure it can be calculated.

YCAS: The ratio of the cytochrome components can vary depending on the genetic background.

GREEN: That certainly is not true for the well-defined organelles. Very precise geometry exists there.

YCAS: I would object to this because yeast or Neurospora can have mutations which will, for example, knock out everything but cytochrome C, and the amount of cytochrome C increases by 50 to 75 percent.

GREEN: Yes, but that kind of evidence is not really relevant to the question of how much cytochrome C is in the organelle. The determination of cytochrome C in the whole yeast cell may

mean absolutely nothing as far as the stoichiometry in the organelle itself is concerned. Most of these studies involve the determination of the total amount of cytochrome C in a cell. Cytochrome C may exist in storage form, and the actual concentration in the cell is not necessarily the concentration in the organelle. The question is this: Do you have variability within the organelle? I do not think there is any evidence that such exists.

YCAS: In Neurospora there is a so-called poky mutant in which the ratios of the cytochromes are changed.

GREEN: In the cell or in the organelle?

YCAS: I have always assumed this is always in the organelle.

GREEN: Ah, that is a very dangerous assumption.

YCAS: Is there any evidence that cytochrome C can exist outside the organelle?

GREEN: It must, I suppose, in some bacteria which can apparently produce it in very large quantities, far beyond the stoichiometry with other components. What I am saying is that when the individual organelle is isolated no evidence for such variability in stoichiometry is found.

YCAS: This is in mammalian tissue?

GREEN: Mammalian or plant or even bacterial systems—wherever such studies have been carried out.

DANIELLI: That cannot really be true because chloroplasts can vary in composition tremendously as far as the lipid components are concerned, and the lipid component is just as important a fraction of the mosaic, so to speak.

GREEN: Yes, I think the lipid is; but lipid is not necessarily concerned with the oxidation-reduction components of the chain. The lipid composition does have some variability and is an expression of the lipid concentration or composition of the cell as a whole. There is that variability, admittedly. It can be changed within limits, but not very much. For example, one class of lipid can never be substituted for another. There is, in fact, a great deal of constancy with respect to the species of lipid molecules, but the question of the lipid is a red herring altogether. Actually, we are talking about the oxidation-reduction components. These are the essential parts of the electron transfer chain.

DANIELLI: Yes, but I do not think we ought to confine our discussion to that because the question raised was whether these organelles would spontaneously assemble if their components came into existence individually.

GREEN: On the basis of what we now know about the organization and structure of the organelles, I would like to eliminate the idea that these could be assembled spontaneously or like parts of a jigsaw puzzle. The spontaneous formation of organelles from the individual molecules is so improbable that I think it is not worth speculation.

Therefore, the question as to whether the parts are made in one chromosome or many chromosomes will not affect the picture. The issue is the type of mechanism required to assemble these things in a meaningful, reproducible fashion. This assembly would appear to require a specialized system.

DANIELLI: I agree with you about that.

GREEN: It is the nature of the assembly system that should be our concern. Where they are synthesized, whether all in one place or many places, may be secondary because there is still the assembly problem, regardless of where they are made. It is not more efficient to

assemble them if they are made in one chromosome. The problem of assembly still remains.

ATWOOD: But it is possible. A series of proteins could be made as a single polypeptide chain with only one N terminal and one C terminal. I think it is unlikely that this happens, but not impossible.

GREEN: "Unlikely" would be an understatement. That is a picture of the membrane which is not a valid one. The picture is one of a sequence of proteins arranged in some linear fashion. That picture does not correspond at all to what is found in the cell because protein and lipid are interlarded, with the protein in varied arrangements. There is as much complexity in the structure of a membrane as there is in the structure of a single protein. If it is assumed that membranes can be put together, it might as well be assumed that proteins could be put together by mixing amino acids. There has to be a high degree of order, which I do not think can be achieved by any spontaneous interaction of parts.

FREMONT-SMITH: Could we say that "spontaneous" means by mechanisms not yet understood?

GREEN: No, "spontaneous" means that no external agency would be required, that just by --

FREMONT-SMITH: In most spontaneous action, has it not been discovered later that there was a mechanism that had not been understood?

DANIELLI: I think that is a philosophical question.

FREMONT-SMITH: I wanted to raise it.

TOTTER: I think Dr. Green has just set back spontaneous generation of life another billion or two years.

YCAS: Is it not possible that it might be a relatively simple repeating structure which will arise, perhaps by some process such as crystallization, and this repeating structure will then have a certain affinity for different components? I do not see why it is totally unreasonable to imagine this.

GREEN: It depends on the simplicity of the structure. If there is a repeating polymeric protein, yes. But where there are a large number of repeating structures, a large number of component parts, and an infinite number of possibilities for arrangement—and only one unique arrangement—then I think it is difficult to talk in terms of spontaneous interaction leading to a particular species.

YCAS: But the number of components might really be small.

SZENT-GYORGI: I would not accept Dr. Green's statement that mitochondria could never assemble spontaneously. I can see many ways in which they can assemble. I have no evidence, but it is a very great order to say "never." One must think it over twice before he says "never."

A protein molecule is such a very specific and complex surface that one can imagine it linking up with only a certain type of protein and then again with another: thus I can well see the possibility of assembly. If the tobacco mosaic virus can assemble spontaneously, why should not the mitochondria?

GREEN: But suppose I asked whether the tobacco mosaic virus could be reassembled, not from the protein coat and the nucleic acid already formed (that is just a matter of bringing the two parts together), but starting from the much simpler units, perhaps the polypeptides or amino acids, or even the fundamental subunits of the virus. That, I think, would be quite improbable.

What you are saying is that the polymerized coat protein and nucleic acid can be mixed, and these two elements will reassemble to form a virus; but the same thing can be done with parts of such complexity. The electron transfer chain can be reassembled from parts that have been dissociated. But to start de novo with all the component molecular species of the mitochondrion and expect these to reassemble spontaneously to form a mitochondrion would involve a high degree of improbability. I doubt that a unique arrangement of these parts could be achieved by a spontaneous process.

SZENT-GYORGI: That is different. You say a high degree of improbability; from high degree to zero is quite a jump.

McMULLEN: I think we can derive a picture of the way this improbable event could occur from the working molecular models of Professor Penrose at University College, London (ref. 6). He has devised a jigsaw system—in other words, working models representing amino acids and nucleotides made, in fact, from plywood. These have interacting parts representing bonds of various kinds, including high-energy bonds, in which an elastic band is used for reinforcement.

On simply applying energy to these models—in other words, on shaking—they do in actual fact line up, only in certain directions and in very definitive pairs, triplets, and so on. By supplying energy in this way, admittedly in some cases in a confined area that may represent a structural portion of the cell, Professor Penrose can demonstrate replication of protein molecules from a template, feedback mechanisms, etc. He has produced several remarkable films illustrating these models, which I think everyone here should see. They are thought provoking indeed.

POLLARD: Are these published anywhere?

McMULLEN: Yes, there is a publication on this in New Biology (ref. 6).

ENGELBERG: The Scientific American also had an article on this (ref. 7).

MOROWITZ: There is a general issue here in the construction of organelles, which, I think, is one of the important generalizations of biology, that is, the uniformity of membrane structure. I think Dr. Green was stressing the diversity of membrane structure, but it seems to me that there are at least three features of membrane where a surprising uniformity exists. One is from electron microscopy, where there is a fairly narrow range of what is seen in the scope—given a standard fixation and staining technique. This is the unit membrane that people now talk about.

In addition, the capacitance per unit area of all the membranes that have been measured (and this is quite a wide variety of cells) lies between 0.5 and 1.5 microfarads per square centimeter. The limited data I have seen on gross chemical composition of membranes also suggests a great similarity. Therefore, I wonder if there is not really a kind of important generalization around the corner.

ATWOOD: You are faced always with these alternatives when you see such a uniformity: Either it is historical accident—that is, it is uniform because once successful and this system will continue to persist as other things evolve—or else it is a necessity for subbiological reasons.

You have the same problem with the appearance of flagella. On cross section they are all alike, no matter where they occur—nine "5-er's" in a circle and two in the middle. Is it because this is the only way one can be made, or is it because the first one was made that way and it has just been inherited from that time on? I tend to think the latter is more likely, that these uniformities indicate common ancestry rather than a physical necessity.

PITTENDRIGH: This idea can be extended even further. Why is adenine present in so many essential constituents in the cell? Is it because adenine alone can perform a certain chemical-physical prerequisite in these molecules, or is it simply because, given the available bases of purines in the cell, we continue using adenine while there may be half a dozen other ways in principle?

I had one other question I wanted to ask, which is one purely for information on this discussion of assembling proteins and then obtaining the solid state, the appropriate solid state array afterwards in mitochondria. It so happens I was discussing this with an elementary class a little while ago, and it occurred to me that I did not know of any gene having been mapped on a chromosome that was responsible for any of the enzymes in mitochondria.

YCAS: There are. In yeast, structural mutations of cytochrome C are known. Fred Sherman in Rochester has quite a number of them (ref. 8).

PITTENDRIGH: That mapped?

YCAS: Yes.

PITTENDRIGH: He is bound to have mutants. The question is whether they are nuclear.

LEVINS: With Drosophila experimentation in the laboratory where there is intense selection conditions we can get a rate of change, at least over shorter periods, of something like 100 000 times faster than the equivalent rate of evolution in nature. This is without knowing anything about the underlying physiology. Therefore, with the appropriate manipulation it might be possible to try to obtain intense selection using unusual biochemical components and let the cells tell us how different they could be.

ENGELBERG: I was thinking that the basic issue here about the way mitochondria are assembled really has more basic meaning in biology. It seems that it is the old question of precision versus just throwing things in a bag to automatically form various parts. Historically speaking, the pattern has been to assume an unprecise mechanism. Thus, at the turn of the century it was believed that a cell was just a container in which chemical reactions took place in solution. As more discoveries were made, we found instead that the fundamental processes were regulated with great precision. Thus, in a discussion on the assembly of mitochondria, past experience would suggest that we should be very careful about placing too much emphasis on the possibility of components spontaneously coalescing.

A question raised by Dr. Friedenberg during the recess has to do with the basic assumptions about what set of physical laws we are entitled to use—the question of localization, for example, that Dr. Morowitz raised here. I wonder whether, at this point in the conference, it might not be worthwhile for someone to say a few words about his thoughts on, for example, Elsasser's point of view concerning what physical laws to use at what levels of biological thought (ref. 9).

POLLARD: I, personally, do not have any comment on that. I feel somewhat out of my depth here. I do agree that it is possible that one more physical law remains to be discovered; and this will be found in biology and, perhaps, elsewhere also. But the only mechanism I know to find such a law is to try everything else and then look very closely at the failures, if any. If we do not find any failures, we do not need any more laws.

The laws that I privately have been thinking work are the laws of quantum mechanics and the laws of electricity plus the additional law of evolution, which is the one biological contribution. These are the laws I believe should apply and should work.

PITTENDRIGH: I may not be entitled to ask this question because I was unavoidably late this morning. However, I am a little perplexed about the title "De Novo Cell Synthesis," because we know nothing about this.

Another point relates to this mitochondrial discussion; there is no de novo mitochondrial synthesis, either. Surely at the very heart of this business of how things are ordered is the fact that the cytoplasmic organelles are remarkable in that they do not arise de novo and that presumably they contain their own ordering principle within them.

GREEN: Do tell us about it. This is an interesting comment. How do they arise? You say not de novo?

PITTENDRIGH: No, all mitochondria arise from preexisting mitochondria.

GREEN: That is news to me. What reason do you have to say that? Is this notion part of the mythology of this field?

PITTENDRIGH: There certainly is very good evidence for the chloroplasts.

GREEN: But let us talk about mitochondria and see what we can make of this.

DANIELLI: Would it not be better to wait for this discussion until Dr. Slonimski arrives. He has quite a contribution to make to this, if I understand correctly.

FREMONT-SMITH: I do believe Dr. Pittendrigh asked two questions which have not been answered, and I think some reference should be made to de novo cell synthesis. In other words, let us briefly review the discussion this morning to bring him into the picture. I think it would be good for us all.

POLLARD: The only thing I said this morning was, when we speak about de novo cell synthesis, is it conceptual? One can imagine a way in which amino acids, nucleotides and DNA can be made; and one can draw on this all the way through until, essentially—if not in the laboratory, then in the mind—there is no step which could not be done.

Now, this will mean, certainly, the synthesis of a mitochondrion from some parts of it; and it would mean the synthesis of those parts from something further back. Or it would mean the discovery that we do not need to synthesize. This is like a salt crystal: Put the salt there, and wait, and it occurs. This we did bring up—the idea of putting three polymerases together in a jar and, boom, it would work.

MOROWITZ: The problem I want to raise about de novo cell synthesis is the problem of the level of organization, which I think has been confusing us all morning. Each of us says "cell synthesis" and thinks about the cell he is working with; yet these are vastly different in order of complexity and, even, in order of magnitude and size. I want to stress this: The mitochondria that we talk about are larger than the E. coli that Dr. Pollard talks about. Indeed, these mitochondria are as large as 1000 of the cells I am going to talk about; we could put about 1000 PPLO into a mitochondrion.

I think this difference of level is rather confusing. If we talk about de novo cell synthesis, I really think we would be on the outer limits to worry about synthesizing a cell with a mitochondrion when the mitochondrion is probably more complex than the total cell we want to think about synthesizing.

DANIELLI: Larger, not more complex.

MOROWITZ: If Dr. Green is right, it is more complex.

GREEN: No, what I want to say is that the size of the mitochondrion is almost irrelevant. We can imagine the mitochondrion being smaller and smaller and smaller until finally we reach the point where we have just a segment thereof (which we could have in bacteria) but, in kind, no different from the more complicated, larger structure because it is a repeating unit.

Let us say a mitochondrion may have 50 000 of a particular type of particle; it could have only 10 or 5 and still be, in gross structural pattern, the same. Thus, size is not necessarily the determinant. It is the qualitative aspect that is important, whether the character of the structure is the same in the smaller unit.

I believe you made a point earlier, which was very well taken; namely, that you are dealing with a fundamentally similar architecture in these membrane systems. That is why membranes look alike and have some compositional similarities. But this, I think, is the common element in all cells. One of the most striking things about the mitochondrion is that whether you isolate it from plants, or even its equivalent in bacteria, or from animal tissue, it has the same type of chemical architecture and composition—extraordinarily similar.

PITTENDRIGH: I wanted to respond briefly again to Dr. Green's question as to what evidence I had in mind. I was thinking that once you lose mitochondrion from the yeast, presumably in the poky situation, you do not get them again.

YCAS: You do not lose them, though.

PITTENDRIGH: In the pokies?

YCAS: In the petites. They still have mitochondria, but the mitochondria lack some of the cytochrome components.

PITTENDRIGH: I was wrong. I thought you actually lost them, which would be analogous to the cases in the chloroplast situation.

POLLARD: I do believe we have a whole day on the organelles tomorrow, and it would probably be wise for us not to spend today on that subject. We are, perhaps, using the time a little unwisely by talking so much about mitochondria.

TOTTER: I would like to raise one question concerning this that I think we have overlooked so far. It would be very instructive if someone would talk about the rates of formation of mitochondria as compared with the rates Dr. Pollard mentioned a while ago for other things. Possibly this would give us a limit which we could put on the probability or improbability of one being assembled spontaneously.

POLLARD: Let us discuss that tomorrow.

DANIELLI: Yes, this is a special item—synthesis of organelles.

HOFFMAN: I would like to make a comment about one of these subjects. About a year and a half ago, I attempted a calculation using a classical mechanical model. With the assumptions that the enzyme was much larger than the substrate, that the forces involved were central forces, and that the enzyme was faced with a linear substrate gradient, I calculated a relative velocity of the enzyme with respect to the substrate. The direction was such that the enzyme moved toward higher substrate concentrations but the magnitude was microangstroms per second.

POLLARD: There is in these channels, I am quite sure, much actual straight "pumping." That is to say, just like a vacuum pump, at one end is built an amino acid into protein, and it is gone out of the solution, thus, there is a concentration gradient that way.

HOFFMAN: This was a sort of pumping from one enzyme to another. For instance, if they are locked on a mitochondrial system, or if one enzyme is followed by the other, it may be possible that the substrate moves automatically in the right direction.

POLLARD: I would like to make a plea for one thing which could be built up as the result of this group's going home and working and, perhaps, writing some papers for Dr. Danielli's journal.

That is, it would not be a bad idea if everybody explored systematically what might be called trivial physical explanations of biological thinking. Pumping by the freezing up of metabolites on structures is one mechanism of transport that might be considered.

Consider Solomon's picture of a membrane as a bowl of spaghetti that is shaking and so opens up pores and lets things through and then closes up again. Such a picture can be subject to physical thinking, and one could actually work out the theory of a membrane in those terms.

If all these sorts of nonliving, purely physical things could be determined as to size and, so to speak, listed in almost an encyclopedic form, it would be very good. Then I think we would find that none of them work, but it would be nice at least to know that.

Dr. Roberts has said that he would like to talk for 5 minutes. He has a rate of synthesis he wants to discuss.

ROBERTS: I would like to talk more about the synthetic rates that Dr. Pollard introduced. The DNA in coli has a molecular weight of 5×10^9 or 8×10^6 nucleotide pairs. It is duplicated in a 1-hour generation time, giving the same rate that Dr. Pollard reported. His was for phage, and the rate is 4000 nucleotides per second in growing coli. The DNA growth rate can be increased by, roughly, a factor of 4 either by virus infection or by putting the cells in a better medium; but in both instances it takes some time before the new growth rate is achieved.

In the virus case there are both more points of synthesis and more polymerase. In the better medium there is only more polymerase; thus, it does look as if this rate might be limited by the supply of polymerase.

Turning to the rates of copying this DNA, Spiegelman* showed 0.02 percent involved in soluble RNA synthesis. This is enough to make 40 different kinds of S-RNA molecules. It develops that these are made at a rate of 2000 copies per generation. The average time required for each is 2 seconds.

The next unit is the ribosomal RNA. Spiegelman* has found 0.32 percent of the DNA complementary to R-RNA. McCarthy and Bolton (ref. 10) find 0.4 percent. I will use 0.4 because that applies to Coli where we know the molecular weight. For 12 regions, each of which can make the full RNA for a 70 S ribosome, 0.4 percent is enough. These turn out 1000 copies per generation per site, and the rate per site is 1000 nucleotides per second.

The rest of the DNA is concerned with making the messenger, or DNA-like, RNA. One strand is read, but not the other. If we plot the genes in their order of activity, so that the most active ones are to the left and the less active ones to the right, there is a very sharply peaked distribution. Roughly 70 percent of the genes seem to be making one copy per generation. About 1 percent seem to produce around 200 copies per generation. The rest fall in the intermediate region. (See fig. 6.)

I think one copy per generation is an interesting number because it may imply that these genes only become active as the point of synthesis passes over them. Of course, these genes can be converted from this type into the fast type simply by induction; thus, there is nothing peculiar about the gene itself.

We now turn to what limits the rate of synthesis. Perhaps R-RNA synthesis is limited by the supply of nucleotides because, on putting the cell in a better medium, its rate goes up immediately. The same number of copies of S-RNA are formed in a generation, regardless of the generation time. We do not know about the variation in the rest of the system but suspect that the total will remain about constant in relation to the ribosomes, although the spread may be broader in a poorer medium.

YCAS: I think Dr. Roberts is considering two distinguishable phenomena. One is the number of times a gene is read per unit time; the other is the rate at which a single molecule is

*Personal communication.

assembled once synthesis is initiated. It is conceivable that the two might be subject to different control mechanisms.

ROBERTS: I think we can be sure that they are all fast, once they are initiated. In particular, if we calculate the synthesis of templates for beta galactosidase, the average rate is something like 18 seconds. But the enzyme can be seen already in 15 seconds; therefore, I believe that it is made at the rapid rate of 1000 nucleotides per second, which would take a few seconds. Then it waits for 15 seconds before it is read the next time.

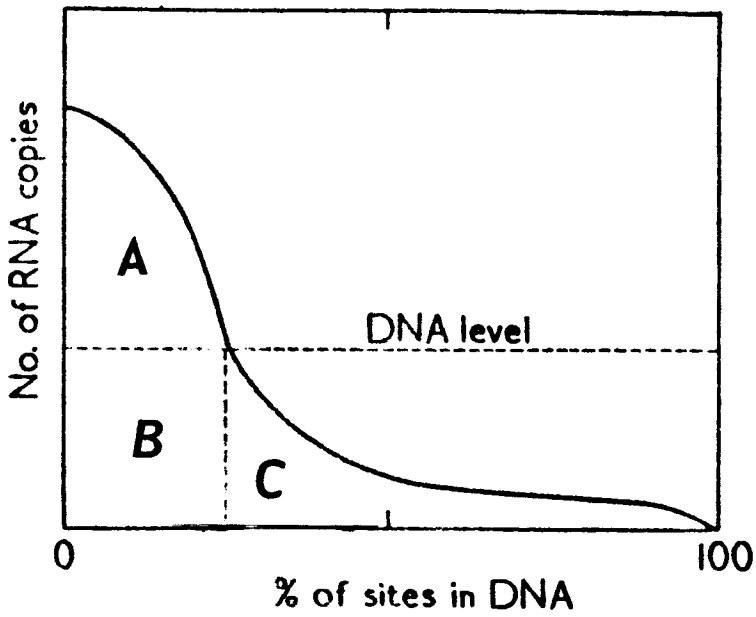


Figure 6.—Genetic sites in the DNA arranged in order of their activity in making RNA copies. The total population is represented by the area under the curve. The DNA may be represented by a horizontal line since, by definition, there are equal numbers of each genetic site. For any experiment the position of this dotted line represents the DNA-RNA ratio employed. Then the area A represents RNA molecules homologous to saturated sites in the DNA, B to RNA molecules adsorbed on saturated sites, and C to RNA molecules adsorbed to DNA sites, of which more copies are available. (Ref. 10.)

DANIELLI: How many polymerases, molecules of the polymerase, are present?

ROBERTS: I do not know that.

POLLARD: That is not too relevant because it is the one strand of DNA that is being read. We do know that one agent, only one agent, is active. There may possibly be 8 or 10 polymerases, so to speak, marching down and leading this stuff off; but it is being read off one strand in any one place. If I understand you correctly, what you are saying is that the ribosomal RNA is being read off at a rate of 1000 copies per second.

ROBERTS: No, 1000 copies per generation; 1000 nucleotides per second per site for 12 sites.

DANIELLI: The point I had in mind was that, while in some parts of the genome the read off is 1000 copies per generation, in other places it is only 1.

ROBERTS: That is right.

DANIELLI: So, it could not be polymerase running the whole length. Perhaps there are many different polymerases that act on separate sections. There might even be some exclusion principle so that only one polymerase could be present on the total strand at a time; but there could be different polymerases at different times. There must be some way of differentiating, at any rate, between 1000 copies per generation and 1 generation. That is the point you are making, I presume.

ROBERTS: Yes, and I do think it is polymerase that limits the rate of RNA synthesis. If a new enzyme is induced, production is started immediately. There is no time to synthesize new polymerase.

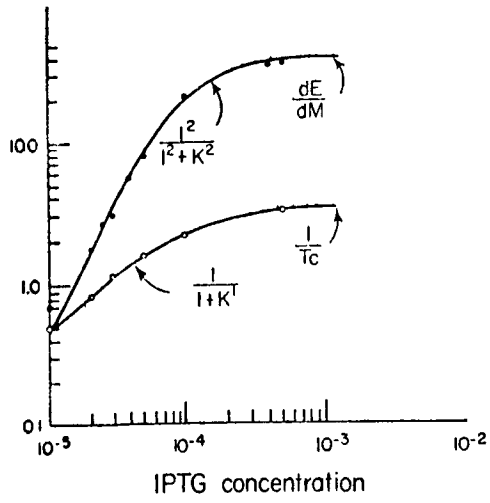


Figure 7.—Log-log plot of the effect of various IPTG concentrations on the steady-state rate of β-galactosidase synthesis and on the time constant. Experimental data presented as circles. Solid circles describe effect on rate of synthesis. The corresponding line is theoretical curve of I^2/I^2+K^2. Open circles describe the effect of inducer concentration on $1/T\sigma$ with the corresponding line that of $I/I+K'$, IPTG concentration is moles per liter. (Ref. 12.)

DANIELLI: It may still be there all the time; it is just put to work.

BAUTZ: If there is just the polymerase, another component must be added to activate it. Then the induction would be that the inducer acts on the polymerase.

DANIELLI: How many molecules of inducer are needed to obtain induction?

ROBERTS: Induction is a complicated function of the inducer concentration. It has a saturation characteristic. I think you have full induction at about 10^{-4} molar.

DANIELLI: How many molecules actually have to get into the cell to do this?

ROBERTS: Oh, a great many. I do not have the exact number in my head, but it is millions.

YCAS: Except penicillin, where not more than 80 is necessary.

POLLARD: Eighty molecules of penicillin will induce what?

YCAS: According to Pollock, (ref. 11) that is the number necessary for the continuance and maximum production of penicillinase by a single cell.

POLLARD: How large is that cell?

YCAS: It is about the size of E. coli. I do not know exactly.

ATWOOD: But that is a case where induction is irreversible, in contrast to the induction of beta galactosidase.

DANIELLI: But why are all these molecules needed?

ATWOOD: The equilibrium between repressor-bound inducer and free inducer for z-galactosidase is far in favor of the free.

DANIELLI: This means the site at which the inducer combines does not combine with it very strongly.

ATWOOD: Right.

DANIELLI: This is odd, because it combines very specifically, but it does not combine very strongly. How can we differentiate?

ROBERTS: I can draw the actual concentration dependencies (fig. 7). If we induce with the maximal level of inducer and at low level, two things change. One is the slope; the other is the time constant. If the rate of enzyme production is plotted against inducer concentration, the curve goes up and saturates; but initially it has a slope of 2, indicating a two-molecule step. The time constant has a slope of 1. It takes quite a series of equations to obtain these two different inducer dependencies—one dependence for the rate and another for the time constant of induction (refs. 12 and 13).

DANIELLI: That time constant is not a time constant for permeation into the cell?

ROBERTS: No. This is done with an ML3, which is so-called permease negative (refs. 12 and 13).

B. FUNCTIONAL STRUCTURES

Discussion leader: F. Forro

POLLARD: We are beginning to move into the conference a bit, and I think we should now ask Dr. Forro to take over on whatever he wants to talk about. I would like to think that this afternoon, when we get to Dr. Morowitz, we will still have enough time so that this whole thing can go around. What we have to remember is that soon I am going to be chairman of a committee that designs the cell. In the program there is a round-table discussion; and since we are going to put the cell together, we do not want to waste our time on frivolities. I will now turn the meeting over to Dr. Forro.

FORRO: I am certainly not Dr. Pollard in respect to theoretical biology, as indicated last night, and you would be amazed if I tried to lead the conference as if I were. Perhaps it has been stimulated enough, however, to proceed in essentially the same way, with individuals asking questions as they have already.

I mentioned last night that I was interested in de novo cell synthesis. I have always had faith that some day, hopefully within my lifetime, someone would be actually able to bring about what has been denied as possibly occurring here; that is, to produce a coalescence of molecules, presumably macromolecules, having the general features that we might consider to be living. That faith has been alternately shaken and reinforced over the years. There is very little to go on except to examine cells as they exist in order to obtain our clues.

We always break down on the idea of resynthesis. Things seem to be too complicated when we start looking at the individual parts to be able to put the whole picture together by starting with any one item that we think we have some understanding of. However, this does not shake my faith. But I still think that possibly we will not accomplish the whole thing because we must recognize, as has been said this morning, that the cells we are dealing with may have been built with something that we do not see at the present time. If we start resynthesis from constituents, we may never be able to do it because parts of the system were made in some way, possibly back along the evolutionary scheme by some machinery which has been dispensed with, which puts in information that we cannot see just by looking at the current sample and trying to make a cell sort of coalesce from current ingredients.

Nevertheless, I think the only way to proceed is to assume that this may not be so and to keep examining and probably to keep prodding ourselves once in a while to ask: "With what we do know could we, in fact, specify conditions where we could have the individual ingredients and bring them together in some way that they would produce the essential thing—a separate physical entity which would multiply, making use of the ingredients within the medium to build materials of itself?"

I think this is what I mean by de novo cell synthesis. I will be happy only when I see it accomplished in a test tube and not just a theoretical accounting on paper of how the biological system now behaves. I believe that the experimental reconstruction is the necessary demonstration in connection with reality of a theoretical conception.

Earlier, it seemed to me that, when we looked at cells, one of the things we were struck with in biology is that cells are cells because they have a certain stability. In multiplying, it is stability that is characteristic of them, although it is the tendency for geneticists to think of change as being the characteristic thing. Change is a rare event which gives them their long-term ability to survive and to be selected for and to evolve, but the division-division feature is, in fact, their stability. Then, in the late forties or early fifties, it seemed to me that there were two ways of thinking about this process. One way was that there exists a beautifully systematized, coordinated group of reactions having a cyclic course: every time a new cell is produced there is essentially a recapitulation of the cyclic set of reactions that

had occurred in a previous generation with the possibility of little or no molecular continuity for any given molecular species.

The other way was that there actually is material in the cell that serves in the current notion of a template. It provides the source of information that the cell uses for what it is, and it also provides the continuity for the next cell when one cell becomes two. This led to asking "What could this be?" It was not difficult to guess, even back in 1950, that DNA was a likely candidate. Thus, the question, "Is DNA a material that is molecularly conserved?" was the one I found myself asking. I thought this would be an essential point to know if one were going to think about de novo cell synthesis, because at least one would not want to start off on a completely wrong tack. Possibly there are many ways to construct systems that a biologist might consider living out of all the material existing in the world, but I think we would like to take our clues from living matter, at least. Therefore, it seemed appropriate to ask this question of the current living matter.

This question has involved me in details of DNA's behavior. I probably operate with some prejudice because I think we have to know something about its replication mechanics before we are really going to get very far because DNA seems to continue to be the central item that has to be replicated before the organism replicates. Therefore, it would be appropriate for me to tell what I think I know about bacterial DNA.

Perhaps I should start off by posing a slightly different view of a bacterial cell. I will put it up as an electron microscopist sees it, and this view has always posed a problem as to whether the electron microscopist is seeing it right. Dr. Pollard looks at it at a much more microscopic level than I.

A bacterial cell is an object having an outside cell wall, quite a heterogeneous material made up in some part by subunits that have been characterized and that seem to have some relationship to the bacterial shape. That is, if the cell wall is taken off a bacterium, it is a spherical object; in other words, it is not the internal structure but the cell wall that gives the bacterium shape. Inside the cell wall there are characteristic double-walled membranes (as indicated by the single line in figure 8).

There also seem to be two phases in the cell: one that is relatively electron transparent and one that is relatively electron dense. In the electron-dense phase there is granular material that in some cases looks to be about the size of materials that have been isolated from cells and called ribosomes. The electron-transparent phase has a checkered history. In the early days, I guess people saw irregular electron-dense material in a mostly electron-transparent background.

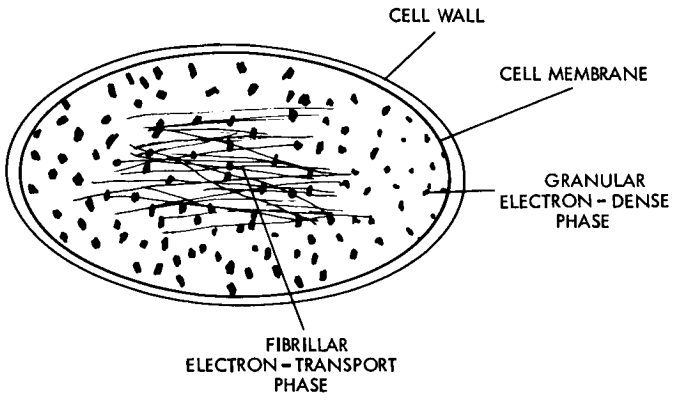


Figure 8.—Schematic view of a typical bacterial cell.

As time has gone on, it seems that people have learned how to fix the cells, presumably to preserve that region, and they now see it as either a disorganized group of very fine fibrils, somewhat as in figure 8, or they see it as considerably more organized, with regions where many fibrils can be seen, somewhat related to each other in sweeping arrays that may curl around in various parts of the cell.

Several years ago people were inclined to say this was DNA. They looked at it and it looked like DNA, so that seemed reasonable. Then we were working with tritiated thymidine in studies on chromosome replication, and it seemed

reasonable to ask the question, "Is, in fact, the DNA localized in this kind of picture?" Van Tubergen and Caro (ref. 14), then graduate students at Yale, undertook the sectioning of bacterial cells which were labeled tritiated thymidine. From studying both serial sections and random sections, they were able to correlate the extent of that region along the length of the bacterium with the number of sections that were labeled. They made a case that this is the region where the thymidine is located, which means this is the region where DNA really is.

The reason I got on this theme is that I think this is the way we think about it. The cytologist tells us there are nuclear bodies in there, and he bases this belief on the old days' evidence that things can be seen in the light microscope on fixed preparations which are appropriately stained. With the electron microscope this seems to be borne out on fixed preparations, and so we may be very happy with the situation.

I think we have only one possible reason to say that this is the way it is in the living state. Possibly somebody may know more than I do, but the only thing I can say is that people have adjusted the refractive index of living bacteria to that of the suspending medium so that they presumably can see the nuclear bodies inside of the bacteria (ref. 15). They say that they do see objects inside which correspond to what they would see if they took the same cells and stained them. This is the only evidence that DNA in the living state is really packed that way, that one has not produced a distortion electron microscopy, and that the DNA does not really penetrate throughout all of the structure. I do not know whether there are comments on this point or not.

MANILOFF: We have studied one of the pleuropneumonia-like organisms and have been able to isolate the nuclear material containing the cell's DNA. The ellipsoidal morphology seen in the cell is retained in our procedures. This indicates that the DNA packing is not completely dependent on the physical chemical environment inside the cell.

FORRO: It either holds together or returns to a compact form. It might do the same thing when isolated as when it is being fixed.

I do not know how much this volume represents close packing of the DNA, but it is certainly material that looks like it. It has to do a lot of functioning—functioning and replicating in a much narrower region than the whole cell volume from the picture seen in the electron microscope.

The question is, "Is the DNA packaged the way it looks in the electron-microscope or is it not?" I can point out my view of the problem, which may be contradicted now or later. I think from there I will move on to the replication story as I see it. One lesson I learned from this kind of consideration was that I do think I can be sold, on cytological evidence alone, that one could talk about discrete structures in bacteria and that nuclear body did not necessarily mean a discrete packet of DNA with a certain amount of information, but might just represent some localized concentration within the cell of DNA of a generalized sort without any stoichiometry in terms of information.

POLLARD: I would like to inject a sour note here. The electron microscopist always draws the picture but never says what he did before obtaining it. Would you mind explaining quickly what you have to do to get that picture?

FORRO: That is the point, I guess, that I am making. I am no electron microscopist, and I will probably miss a step. We usually fix them in some way, which means taking the bacteria in this case and putting them in a solution of something that precipitates proteins, at least, and, hopefully, also precipitates other materials. These actions of fixatives, as far as I understand it, are receiving some study. I think the electron microscopists are moving in the right direction. At least, they are examining this question of what fixation means in terms of individual molecules, and I think that is a fair way to go about it. But the presumption is that

this fixes the material, the biological section, the way it was in the living state before precipitation.

GREEN: That they do not know. They hope that that is so.

FORRO: That is the basis for this operation.

GREEN: That is right. They assume, or would like to believe, that that is so, but they do not know.

FORRO: That is what I mean when I say they are coming in the right direction. I see in the literature many, many reexaminations of the question undertaken by building artificial systems, taking collections of protein molecules or DNA, and actually asking, "What does it look like if we take a collection of molecules and actually fix them?" I suppose the idea is that the protein is denatured but left where it is.

POLLARD: But denaturing a protein is not the same as precipitating it. To precipitate a protein, it must be aggregated. The light scanning property of that protein will be materially and violently altered.

FORRO: Perhaps I used the wrong word.

POLLARD: Therefore, the aggregation of that protein cannot possibly be the same.

GREEN: There is another point to be stressed that is relevant to the methodology of electron microscopy: that is the practice of introducing atoms having a high electron density. The high density is not inherent in the structure. In fact, there is so little difference in electron density that, unless stains of high electron density are introduced, little structure can be recognized.

The other question is, by the introduction of this new molecule, be it osmium or anything else, what has been done with the preexisting structure? This is utterly imponderable at the moment.

FORRO: I feel that I ought to call upon you to expound upon electron microscopy since I am not an expert. If you, or somebody who knows more about this, can answer the question better than I, please do so.

GREEN: I am not an electron microscopist, but I think Dr. Pollard made a good point in saying that the pictures seen in electron microscopy may be very far removed from the state structures of the cell and that there is a great deal of interpretation involved in making the transformation back to the cell.

I think one of the most exciting developments in recent years is the introduction of reagents like phosphotungstate (ref. 15) which are, on the whole, much milder than anything that has ever been introduced; and some of them do not denature some proteins. There is a possibility that the new structures emerging in electron micrographs obtained in the presence of these milder reagents will have a greater validity than those structures seen in electron micrographs obtained in the presence of reagents that are really terror reagents, such as osmium tetroxide. One does not really know how valid those structures are.

It is interesting that phosphotungstate has revealed order and structures such as had never been seen with osmium; thus, there may be a whole new world emerging when the mild reagents become more generally used. Much of the classical electron microscopy is based on structures arrived at by these very violent reagents, relatively speaking; and it is highly problematic that the picture of the cell emerging from such electron micrographs has much validity as far as fine structure is concerned.

STARR: I would say that the extent of alteration toward mild treatment as between osmic acid and phosphotungstate is probably negated by the further handling which these poor microbes undergo in the preparation of thin sections. This involves, as in all histologic procedure, a dehydration, passing through various alcohols until finally the material is sufficiently dry so that it will not precipitate the plasticizers used for embedding. After a period of time this cooked, dehydrated creature is then cut. If it is cut, these things are seen, but an awful lot has gone on in between.

GRENELL: This does not actually have to be done.

GREEN: Not with phosphotungstate. Such procedures can be cut out. It must be dehydrated, of course, but it is not necessary to subject the specimen to solvents, and certainly not to sectioning.

MANILOFF: What is the reference for the use of phosphotungstate in studying cells?

GREEN: The original work is contained in the paper of Brenner and Horne (ref. 16).

MANILOFF: But these are all in viruses.

GREEN: The method has been used by Fernandez-Moran (ref. 17), Smith (ref. 18), Stoeckenius (ref. 19), and others for the study of whole cells or organelles.

McMULLEN: This is the negative staining technique you are talking about?

GREEN: Yes.

MANILOFF: There are several alternatives to osmium tetroxide fixation. First, there are the techniques of freeze drying and freeze substitution, which are less traumatic than osmium tetroxide fixation.

GREEN: They are mild, anyway.

MANILOFF: Second, there is the class of mono- and dialdehyde fixatives, formaldehyde being the most obvious example, and glutaraldehyde currently being the most widely used (ref. 20). With these fixatives, ultrastructure is preserved about as well as with osmium tetroxide. In addition, however, enzymatic activity is retained, opening the area of enzyme cytochemistry to study.

Therefore, wide varieties of fixative techniques can be used. But the power of osmium tetroxide preservation of structure is so great that all other methods are measured in terms of how well they compare with it.

McMULLEN: I think considerable doubt is being thrown on some of the interpretations of osmium staining observations. It has been shown that in the case of bimolecular leaflets of phospholipin the osmium, in effect, is much more highly concentrated in the region of the hydrophilic groups than in the double bonds in the interior of the membrane. This throws a completely different orientation on the interpretation of these structures from osmium density photographs. Therefore, one has to be careful here, I think, in just assuming that osmium goes onto the double bond preferentially to other regions of the structure.

FORRO: I think I will move on to the general topic because the point has been made as far as bacteria are concerned. I will now talk about the replication and, hopefully present some evidence to provoke further thought! There are, in fact, discrete objects.

When I started studying replication, I wanted to ask the question: "Is there large-scale conservation of nucleic acid material in the course of the replication act in bacteria? And if there is large-scale conservation, to what extent? Is it one unit, two units, three units, four units or whatever?"

The simple kind of experiments which I have been playing with for a long time and just extending are to grow bacteria in radioactive thiamine; these are thiamine-requiring bacteria. Perhaps it would be worthwhile to discuss the precise organisms because I think the sequential questions brought up this morning may not be the same from one organism to the other.

Most of the work I have been doing is with E. coli, strain 15. I work mainly with a log-phase population of this organism. By this, I mean a culture growing actively in minimal medium (just mineral salts and water with the necessary carbon source, usually glucose) at titers of less than, or equal to, 2×10^8 per milliliter. We can take cells grown for a long time in tritiated thymine, wash them, put them out on agar blocks on a microscope slide, let them grow, and then subsequently autoradiograph them.

We can superimpose photographic film upon these cells. Incidentally, they are fixed in a way that probably does drastic things to them, but we do not care in this instance as long as the content of the cell stays in the cell. The whole preparation is put away for a time while the radioactive atoms that have been incorporated decay. After developing the film, we can look at the pattern of radioactivity over the individual cells in such a microcolony; if things are very simple, we might see something rather dramatic.

It develops that things are kind of dramatic, but they are not so simple; thus, we have to hunt for the simplicity. What we see are grains distributed in the emulsion, which we can look at simultaneously with the colony because we do not remove the emulsion from the colony.

We see grains in various patterns. It might be a typical colony that we would see. If anyone wants to look at them, I will just circulate a paper (ref. 21) that I have here.

Therefore, faced with this heterogeneous situation, the natural thing to ask is that possibly this does not reflect the fact that there are many separate objects in the bacterial cells, that they are all different cells, and that they separate out rather willy nilly. Perhaps it is simpler than that, but there are two processes involved. There are objects that naturally want to separate from each other because they have a tendency toward conservation when they replicate, but they have some other feature superimposed on them. The natural thing is to borrow from other experiments and other organisms and think of this as possibly a crossing-over process occurring in the course of the replication that disperses the label somewhat.

Therefore, we start to hunt for the simplest situation. In a log-phase cell we see that there are colonies like this in which there are four packets, not necessarily equal in size, and we cannot find anything simpler than that. This is disturbing because, about the time we are doing this experiment, other people come along, Meselson and Stahl (ref. 22) to be specific, and do a beautiful experiment looking at DNA replication where they take the molecules out of cells and follow the density of the molecules as they replicate.

I do not know whether all are familiar with this, since this is a heterogeneous group, but let me say that the conclusion of this experiment is that molecules harvested from cells, which are on the order of size of 2×10^7 of molecular weight, behave as if they are made up of two subunits having a semiconservative type of replication. That means that the individual subunits form a new partner as they replicate and then separate from each other with their new partners. Thus, when we see that DNA molecules behave as if they were constructed of just two subunits, we begin to ask ourselves questions about the biology of E. coli.

One of the first questions raised is that of the continuity of DNA synthesis. At that time it was not known whether or not DNA synthesis went on continuously in bacterial cells. Some work has been done in our laboratory (ref. 23) and in others in Europe (ref. 24). The general conclusion is that DNA synthesis is, in fact, continuous and that, unlike higher organisms, there is not a period devoted to DNA synthesis when chromosomes are made and then divide but, in fact, DNA synthesis seems to be going on all the time. To try to find a gap in that

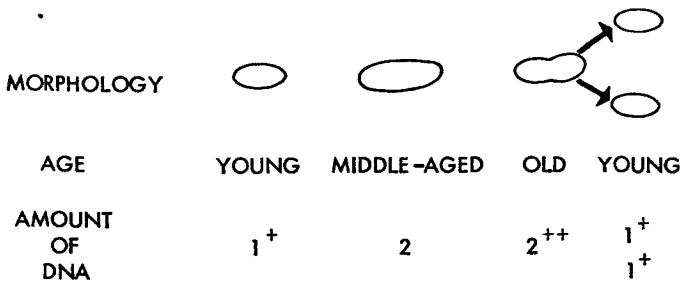


Figure 9.—Hypothetical relation of bacterial morphology and DNA contents during a division cycle.

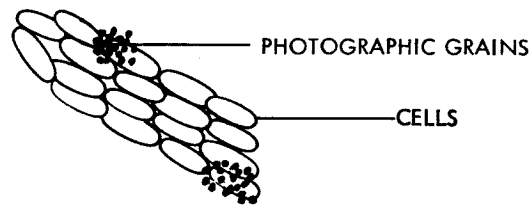


Figure 10.—Autoradiographic appearance of colony with two labeled cells.

synthetic pattern certainly reduces the figure to very, very small times; that is, on the order of 1/100 of a generation time, at least in the normal log-phase cycle.

Therefore, the question then becomes: What are the DNA packets in the cell, and how are they related to the molecules derived from them and to the patterns of continuous DNA synthesis? We decided to adopt a model to see where we could go with it. Figure 9 depicts a log-phase cell as it goes through its growth cycle. This is one which has just recently divided, goes on, and grows longer as it goes on—it has an indentation in it—and, finally, it becomes two daughters.

I am not being interrupted but there is obviously something wrong with this. However, I will go just a little further. We could take the point of view that this cell (the youngest) starts out with a simple DNA structure—that is, a single unitary genetic structure when it is just divided—and it will grow. It has to get to 2 somewhere, and we could say it gets to the 2 at the point where it divides.

But if individual molecules look semiconservative, why do I not see semiconservative as the simplest case when I do an autograph experiment of this kind; that is, fully labeled? Why do I not see just two objects coming out of the cell at least sometimes? Perhaps that is not the right way to view it; perhaps the way to view it is that young cells do not necessarily contain just one genetic object. Cell division may not necessarily occur just when the chromosome has finished duplication. Therefore, we can modify the model and say that duplication arrives at some time in the middle of the cycle. This cell (old cell) is 2^+, and then it comes over to 1^+ (young cell).

Taking this view, then, experiments were done in which a label was put in for a fraction of a generation time. Under these circumstances colonies were obtained which had just two objects labeled (fig. 10). To circumvent the problem that possibly this is only part of the total minimum packet seen, since labeling occurred for only a fraction of the generation time, we also decided to take advantage of something else that was observed. As bacteriologists know, we have learned that cells do not retain a constant morphology, even in the same growth medium, as they grow to higher cell concentrations. They shorten up. What does this shortening up mean? The view is that probably they are coming closer and closer to wanting to divide when the DNA packet is doubled; thus, the young cells are actually coming down on this scale and getting closer and closer to containing one DNA unit.

If this is the case, when cells become smaller as their growth rate slows down, even with fully-labeled cells we can now find colonies with only two labeled packets of DNA. Furthermore, if we do the experiment right, we do not see colonies that have only one packet. This gives some validity to the model that, at least for the simplest structures in this population, we can make the proposition that there are structures which would replicate in a semiconservative fashion and that these structures are large. They are large compared to the

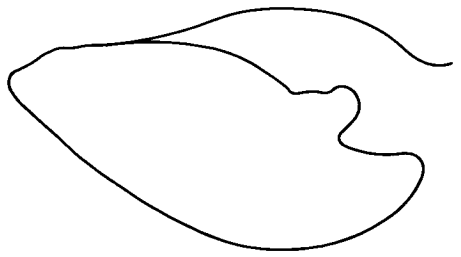


Figure 11.—Autoradiographic pattern from isolated labeled E. coli chromosome.

individual molecules obtained from the cell into a test tube if we were trying to handle them in the gentlest way that we know how at the present time.

This view, then, that the simplest cells would contain a single chromosome which is, essentially, a single object, a DNA structure which has two units, has certainly been borne out by other beautiful experiments. Cairns' experiments were mentioned this morning (ref. 2). Cairns has succeeded in very gently lysing cells of populations like this and spewing out the DNA from these cells in such a way that DNA unravels. It unravels on a cellulose membrane, and he then makes an autoradiograph of the membrane. In some cases he can see autoradiographs of structures which are almost continuous and very large-structures similar to that in figure 11. If we measure the linear continuity, we can get linear continuities giving molecular weights of the order of 2×10^9 molecular weight units.

If I try to make estimates of the molecular weight of the two structures seen in colony autoradiographs from estimates of the efficiency of the film and so forth, the best estimate I can come up with is the same—about 2.4×10^9. Note that it is a little lower than what was put on the board a while ago. This is possibly more relevant to the soluble RNA story, where it has been estimated that there are more types of soluble RNA than the number of different amino acids. It looks to me as though it is closer to the right number.

STARR: I can suggest an organism that has a more extreme condition between the younger and the older cell—a bacterium called Arthrobacter. Arthrobacter, as a young organism, is 1 micron by about 4 micra. It has a regular cycle of development in which it probably becomes multinucleate. Each organism divides into several smaller organisms. This could be very instructive since we now have a bacterium with possibly four times as much DNA in it as E. coli. It would be interesting to determine the amount of DNA in each of the new daughter cells. This is cyclic and can be followed as such if the coccoid daughter elements have a factor of one placed around the upper limit.

FORRO: I think you are speaking of a general proposition that this method could be used to sort out the nature of intricate structures that might have components maintaining their integrity upon distribution to progeny.

STARR: Even with a greater spread between the just predivided stage and the final divider.

POLLARD: I think we want to be a little careful. It is fine to suggest better experiments. But to my understanding what has happened is that now we have two plus lines of evidence which say that, in the cell, something like 2 billion molecular weight DNA is present in one unit. Therefore, in a certain sense we are already far ahead of electron microscopy because we know we have put that in the cell. Thus, we can start putting it in the cell and deciding where it can possibly go, and we do not have to worry about it. For one thing, we are fairly certain that the smeared odd region is not going to be there because we know the DNA is somewhat springy; if it is rolled it will tend, therefore, not to form an odd shape. We are fairly certain of that. Thus, again we are already far ahead of electron microscopy.

What I would like to ask is this: What further evidence to you think you can bring out of these studies here? Is there any evidence that this is actually a Watson-Crick structure, or are there two Watson-Cricks or four Watson-Cricks or anything like that?

FORRO: You have to tie that to the Meselson-Stahl (ref. 22) result.

POLLARD: Never mind whose experiments. What is your feeling?

FORRO: If you want a Watson-Crick structure, I think these autograph results (figs. 10 and 11) bear this out as much as anything. If we miss on our numbers, perhaps Cairns (ref. 2) is seeing half or some other fraction of what I see. Cairns' result has more to it than just the display. It has an analysis in terms of grain densities at structures like this Y. As I indicated this morning, I am not entirely happy with the broad-scale conclusion that his results are indicative of strictly sequential synthesis in all the organisms in the population. They are certainly consistent with the idea that the displays he discusses are Watson-Crick structures, and the evidence is that the densities of the DNA strands are reasonably consistent with this interpretation.

BAUTZ: On the macroscale I agree that the evidence is overwhelming that the replication of DNA is sequential, but DNA is antipolar. There are a 3' and a 5' end on each end of the DNA molecules. To incorporate the deoxynucleoside triphosphates, it seems that free 3' hydroxyl ends are needed (ref. 25). This is perfectly all right if we start duplicating one strand from one end, but what about the other one? The replication of the second strand should go in the opposite direction. The only way to reconcile such a mechanism with the observed unidirectional synthesis (ref. 26) would be to make the assumption that, as the synthesizing machinery goes along, the opposite strand is replicated backwards, piece by piece, involving many starting points (3' hydroxyl ends).

FORRO: I am not sure this is so.

POLLARD: Is it clear what the problem is? This is an important problem that Dr. Bautz has brought up: the mechanism that Dr. Cairns (ref. 2) showed in his radioautography on replication where the Kornberg polymerase does not have the property of having to work with the 3' end at only one place. Now the question is, "How can we reconcile the two?" The suggestion has been made that one strand is essentially either breakable or already broken.

BAUTZ: This could not be observed by radioautography.

POLLARD: Does anyone wish to discuss this? This is an important point because, if it is actually true that one strand is essentially always fragmenting, this destroys a fair amount of beauty in the idea of the Watson-Crick structure. It does one other thing; it makes it easier to bend, and this might be good.

FORRO: The only answer I have to this is that I do not think we know whether a polynucleotide triphosphate could be added to a monophosphate at 3' on the monophosphate and perpetuate a triphosphate structure which is continually adding that way with that enzyme.

BAUTZ: Right.

FORRO: Therefore, if the enzyme could—this is a possibility—I am a little surprised that biochemists have not done this. Is there some technical reason why that experiment has not been done?

ATWOOD: What is the experiment?

FORRO: The experiment is to take polynucleotides, make them triphosphates on their 5' ends to see if they can add on the 3' end of 5' mononucleotide triphosphates, perpetuating the triphosphate on polynucleotide chain.

POLLARD: Pushing it out one all the time?

FORRO: Yes, pushing it out one all the time.

ATWOOD: It almost must happen that way. Cairns has a picture showing that all three branches from a growth point are double stranded. Thus, one of the chains must be growing in the direction indicated, with a terminal 5' triphosphate.

FORRO: Is that a published picture?

ATWOOD: I do not know.

FORRO: This is a different type of experiment than he has reported in the literature. It was grown in cold after hot?

ATWOOD: Yes.

FORRO: Dr. Pollard's question to me was, "Why do I think it is this way?" I think Cairns' evidence is quite strong. The radioautographic density for unit length is what would be expected of a Watson-Crick molecule labeled at these specific activities.

YCAS: Then, if I understand correctly, Lornberg's enzyme cannot be responsible for the complete replication?

ATWOOD: This cannot be concluded with certainty, because all the primers used with his enzyme are mechanically fragmented and would not have the triphosphates on their ends.

POLLARD: Dr. Danielli, I believe the following questions that I have on nucleic acid represent something we would all like to know. And I am not going to let Dr. Forro off quite so easily. I thought that if he does not know the answers to these five questions, somebody in the room might:

1. How does DNA coil?
2. Does it have to be separated to be read out?
3. How does it move with respect to polymerases?
4. What, physically, is a repressor?
5. What, physically, is an operon?

Let me first point out that we have one unit of DNA; it has to coil not less than, I believe, 800 times. Disregard fitting the nucleus inside the cell. The question is, How does it do it? Let me ask, in conjunction with that question, does anyone in the room know whether the physical constants of DNA will permit these being coiled? In other words, is DNA sufficiently flexible that we have a Young modulus that will allow us to coil it? Does anyone know the physical constants of DNA? Is there any elasticity parameter of DNA?

MANILOFF: Will you clarify the question? Do you mean how the helix can coil back onto itself?

POLLARD: I want it all.

MANILOFF: You also want to know how a linear sequence of nucleotides from a linear array coils up?

POLLARD: Yes, that would be nice. I do not mean how does it do it of its own accord—how does it generate the coil? No, I am not asking that. How does it actually coil? Imagine that God coiled it; do not imagine it coiled itself. That is another problem; that is an excellent problem, and a good one to think about. Suppose we just took a cell and we had a good enough microscope to look at the DNA—what would it look like?

What I would like to introduce here is that, in terms of theoretical biology, presumably DNA can coil only in certain ways. It cannot coil just up and down. What is the minimum radius of the curvature that is possible in DNA under the circumstances of the cell? Does anyone have any suggestions?

BAUTZ: There is one example I can cite. We have this situation in the phage—how the DNA is left within the phage head. And there the question is, Do we have base pair regions that are not as strong as other ones? Do we have a preferential opening up at certain points? These should then be more susceptible to, let us say, some mutagens; and in this way we should detect "hot spots," which we certainly get. However, it is difficult to correlate the "hot spots" with such a scheme because they occur throughout the rII region.

YCAS: Is it true that the "hot spots" are immediately adjacent to "cold spots"?

BAUTZ: No. The difficult situation is that for different mutagens the "hot spots" are on different locations.

YCAS: It is not a large area which is hot. It is a very small one, right?

BAUTZ: At certain points, even.

MOROWITZ: One thing is fairly clear: from charge-charge repulsion, the DNA probably has to cocrystallize with something else if it is going to coil into the very small spaces where it is usually found.

FORRO: Except that we can draw fibers under circumstances in which there are crystalline regions where the packing comes close. In such cases, is the charge completely negated?

MOROWITZ: I do not really know.

ROBERTS: Spiegelman* had some pictures of the ϕ-X circles, very beautiful pictures. They showed both circles and figure eights, and figure eights looped again up to about eight loops. That showed that it was able to bend around at a fairly sharp radius.

POLLARD: One strand?

ROBERTS: No, that is the replicating form.

FORRO: In the phage head Kilkson and Maestre (ref. 27) at Yale have proposed a model which is at least consistent with their X-ray data and that of North and Rich (ref. 28). The data indicate there is a preferred orientation of the DNA in the phage head. They have made calculations from their model which indicate consistency with birefringence measurements on this page. The model, as produced, involves a bending; that is, taking the DNA and making it have a loop of about (I forget the numbers now) 80 angstroms in diameter where it comes back and interdigitates each time around.

If this is the Watson-Crick helix, it is wrapped around so that it comes back again on itself. The base pairs are perpendicular to the phage axis. Then these things are wrapped in the phage head, with a series of coils running in the center.

The value of this model is that we can conceive of unraveling it simply by rotation about the axis and just pulling it out. It is something like a ball of twine, and it just comes on out.

There are no sharp angles in this model. It is just a continuous structure, and the numbers are reasonably consistent with the fact that there is definite orientation in the direction of the phage axis and the birefringence is of about the right magnitude.

As far as other models are concerned, there are many that can be though of, but none are very satisfactory for meeting the several requirements: X-ray data, birefringence data, the lack of sharp angles, and the ability to get the DNA out.

BAUTZ: If we assume that DNA is circular and if it is consecutive, then we have to have a sharp bend; otherwise we have to break it.

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*S. Spiegelman, personal communication.

FORRO: I do not want to suggest it as a circle. Some evidence indicates it may be a circle. Electron microscope pictures, however, show ends of spewed DNA in which two ends come out of a single phage (ref. 29).

BAUTZ: How big is the tail of the phage?

FORRO: I think people argue about the tail plug and whether the hole in the tail plug is the place the DNA goes through or whether the whole tail plug comes out. That is the whole of the argument: Is the hole in the tail plug about the size of the DNA? If we can take the tail plug out, then we can pull through a monkey strand.

POLLARD: Has anyone else any comments on this question? I think this is very definitely a question for what we might call theoretical DNA scavenging. I call attention to the fact that this problem of coiling must be met by physical constants of some kind for DNA, that these are not known, and that they should be known. Has anyone calculated this? Is no one in the room interested in this?

FORRO: Kilkson and Maestre (ref. 27) tried it, but from a different point of view. They did not have any numbers about DNA elasticity; they just estimated the average bond angle distortion in the DNA back bone for their model and compared this with values of known distortions in simpler organic molecules. The value is not large compared to known permissible distortions. But this does not answer the question, Is it physically possible for DNA? They did not have really good estimates.

POLLARD: Do we not have to write down DNA elastic contents as something we should know about either experimentally or theoretically? We have, apparently, no answer to this. I would just point out that if we are going to wait for electron microscopy to tell us how DNA coils, I respectfully suggest we will wait longer than we should.

Now, what about the second question? Does anyone have any answer as to whether the DNA has to be separated in order for messenger RNA to form?

ROBERTS: I heard Spiegelman again 2 days ago, and he had circles which replicated one strand. When the circle is broken, it replicates both strands. Possibly it does not have to separate.

BAUTZ: Since the RNA is complementary to one strand, I think the DNA has to open up locally; otherwise, we should observe hybridization with double-stranded DNA, which we do not. It is difficult to conceive that RNA is made that way, but it looks as though DNA has a slightly higher affinity to itself than to RNA. Therefore, the DNA, by closing in again right behind the enzyme, should then push off the RNA. I think there is something in the literature (ref. 30) showing that, in the RNA polymerase system, double-stranded DNA turns out single-stranded RNA whereas a single-stranded DNA primer ends up as a DNA-RNA hybrid.

ROBERTS: That means the specificity is placed on the base pairing?

BAUTZ: Yes.

ROBERTS: Does that really give enough specificity? Do we not have to have an enzyme and then we need to open it up?

BAUTZ: You mean the enzyme is then recognizing the other strand?

ROBERTS: Yes, without opening. That is the alternative. I do not think we can rule it out.

WOESE: It is possible to check this point by using agents, such as mitomycin C, that would cross-link the DNA and, thus, prevent its opening at these points. Does functional RNA synthesis occur under these conditions?

BAUTZ: Or actinomycin.

YCAS: There is an answer to that if you accept that nitrogen mustard is a cross-linking agent.

WOESE: Let us accept it without being overwhelmed by it.

YCAS: Apparently protein synthesis and RNA synthesis occur continually when a cell is heavily reacted with nitrogen mustard.

WOESE: Then it is not necessary to open it up locally?

YCAS: I do not know.

POLLARD: There is something here which, I must admit, astonishes me. Essentially, the consensus is that a tube of double-stranded DNA can be just run through an enzyme and it will faithfully copy one strand in units just suitable for a cistron. Do I hear this? Is this what I am being told?

ROBERTS: You added "the units suitable for the Cistron."

POLLARD: That is where it must be if it reads off of a Cistron. I want to be quite clear that this is what I hear in the room because it sounds miraculous to me. This double-stranded wire, or whatever it is, goes through a miracle producer—namely, an enzyme. Now we have a really beautiful biological superstition here. We have put this in an enzyme, and this enzyme "recognizes" the base order as this goes through.

BAUTZ: I do not agree with this thought for the very reason that the single-stranded DNA should not be able to be a primer for polymerase, because then I should think that the polymerase would recognize the base pair and not the particular base.

POLLARD: You do not like that, in other words?

BAUTZ: I would like the idea that they come apart, at least locally. They open up and then close together.

POLLARD: Then, this idea is tenable. If my two hands represent a base pair, these stay together almost all the time, but there is a moment in the enzyme primer structure when they do this (gesturing), this one is then read by some mechanism of the enzyme. Therefore, one of the physical things we have to think about is a process by which coiled-up DNA in the form that is in the cell, going around corners and everything, is continually doing this the whole time, with the result that there is really a wave of this kind of motion going down the DNA at a pretty good rate—in fact, a thousand bases a second, I think. That is definitely an interesting theoretical question: How this can happen—how a molecule can, by any means, do this opening up, basically be copied, and close up again.

FORRO: I think the reason we will not accept is that it goes on to DNA synthesis after opening but, instead, closes again is because, according to Dr. Roberts' comments this morning, certain genomes make a good deal of RNA. But if most of the genomes are making only one, then it raises the question about little localized spots of RNA synthesis, or something like that, corresponding to the microsome.

ROBERTS: Then you would have too much of that kind of DNA.

FORRO: Yes.

MOROWITZ: Except that it may be unstable in smaller pieces.

POLLARD: Can we ask why the readout is necessarily 200 times? Why is it not possible for one enzyme to be made 200 times and another one made only once, so that DNA can be actually

copied only once? Is this excluded? Are there experiments to exclude the idea that that messenger is used in one case and not another?

ROBERTS: The experiments actually measure what messages are present, and we find that one may be present 200-fold and another one singly.

POLLARD: I see. But it is not necessary for the DNA to stop, have five or six copies made of it, and then go on? It cannot all continue one way, but it has something a lot of times?

ROBERTS: Yes. In fact, it must be able to read out at any place, no matter where the point of DNA synthesis happens to be at that time.

POLLARD: Is there evidence that the readout process is random, that DNA synthesis is sequential?

ROBERTS: If readout were not random, we could not obtain full induction immediately.

POLLARD: Therefore, there must be random messenger readout, or approximately random let us say, and not sequential? That is interesting and that, I think, is quite important because it is much easier to see this sort of whole line kind of chain through the cell and then being replicated. This is easy. But it is not easy to see a process whereby this is going on and replicating steadily and uniformly and then occasionally regions tapped to be read out.

To summarize this second question then, we are somewhat equivocal about it. The weight of the evidence supports the idea that, for almost simple logical reasons, there must be a momentary separation although the separation does not involve wide bowing out of strands.

The third question we have answered to some extent is that we said there is a sequential entry of the DNA into the DNA polymerase because this is made in two places—presumably something like pulling a tube out of a tire, as well as we can judge and, presumably, this is a sequential motion around. However it moves, it moves in a sequential way with respect to its own polymerase.

But with respect to the polymerase that will read it out in forming the hybrid RNA, no such sequential motion is at least necessary. In fact, it is challenged on the grounds that induction is so rapid. In other words, something must occur. Instructions must be capable of being given by an inducing molecule to make beta galactosidase at any time in the cell cycle; and, therefore, presumably a machinery for reading out the Cistron must be there all the time—to make beta galactosidase for example. This is a small miracle, and it is interesting.

The fourth and fifth questions are mostly personal, for myself.

FORRO: May I ask a question? In the case of beta galactosidase, have people (I guess they must have) worked with synchronized cultures?

ROBERTS: With the Tau phasing, that seems to work.

FORRO: On induction, does synthesis start right away, even if the cells are at one point?

ROBERTS: They do not start right away; there is a little lag, and then they are all right.

FORRO: Is every cell making beta galactosidase at the same time?

ROBERTS: I think so.

POLLARD: What is the overwhelming evidence of the physical nature of a repressor?

ROBERTS: If there is a verb, there has to be a noun. That is the evidence.

POLLARD: Does anyone know this? I will give you my view, my theory, just to be shot down. There are two or three SRNA's that have the wrong things on top of them, that are not amino acids, that have been put on by mistake.

ROBERTS: There is another hypothesis that it is a piece of RNA, which is DNA-like in its composition, fixed on the DNA. It would have to be small because, otherwise, it would have been found. So it is roughly 1 percent of the DNA and stable. That is something to look for, and it is being looked for.

ATWOOD: Another conjecture is that it is a protein. The logical reason for thinking this is that it has to recognize two things at once: one is the operator region of the operon and the other is the inducer. It is difficult to think of an RNA structure that would recognize an inducer, but easy to think of a protein serving this function.

The Monod model calls for a protein which has two binding sites, one specific for the operator region, which is considered to be a stretch of a few nucleotides. This is enough to make it specific so that it does not recur elsewhere in the genome, but not enough to cause the protein to be too big to recognize it. Then the other site recognizes the inducer. There is a connection between the two sites such that the binding of the repressor to the operator is drastically influenced by whether the inducer is on the other site. This property of one site's being influenced by another has been named; it is an allosteric property, so that any allosteric protein is suitable.

What is the evidence for this? It is the dominance of the I+ repressor G over I- in bacterial zygotes, and to a lesser extent the temperature-sensitive mutant of Novick which is hard to imagine except in the sense that there is a thermal-labeled repressor protein. It is hard to imagine how a stretch of RNA could be any more temperature-sensitive than any other stretch.

HOFFMAN: If we accept both the triplet coding and the sequential making of the RNA, even though we start at different points (possibly in the DNA), then we would need only something to cover up just one or any number of nucleotides. Not multiples of three is three itself, in order to give nothing but nonsense from then on. It could be very small.

POLLARD: I will give you one more or less frivolous suggestion. I will suppose we have a nucleotide that is a phenylalinine, histidine and alanine and I will suppose that this is a group that actually does the work on the enzyme. This group hooks onto the double sugar and does the job. This is just a set of SRNA's that has specificity for binding of the substrate, but it is not an enzyme because it does not have the proper structure to provide the necessary energy of activation for the enzymatic process. In comes the inducer and sees these three things and thinks, "Fine, I have what I want." It hooks on, and this is enough to cause the Brownian movement to get them off. Is this possible?

BAUTZ: Off what?

POLLARD: To get it off the DNA.

What is the overwhelming evidence about the operon? Does anyone have any knowledge of that? These are all ancillary to DNA. That is why we are discussing them. Now I would like to let Dr. Forro off and to let Dr. Morowitz go on.

ROBERTS: May I add one more word about the rotation of the DNA? The rotational speed is about 300 revolutions per minute. That might have an important part in readout.

STARR: Along what axis?

ROBERTS: That is just from untwisting.

C. THE MINIMAL CELL

Discussion leader: H. J. Morowitz

MOROWITZ: I am going to talk from a theoretical point of view about what should constitute the smallest living cell (or the minimum living cell); then I will present some evidence as to what comes closest, experimentally, to this minimum.

First, since this is a theoretical conference, I would like to set forth some biological generalizations. I think the role of theory, if it is going to have any role, is to be bold enough to make generalizations; and I think that at the level of molecular biology a number of these are obvious, but we do not say them often enough or loud enough to really think about them seriously.

I will note five generalizations, although there are probably more that can be made. The first generalization is that the biological information is structural, and this is rather important if we think about de novo cell synthesis. What I mean by "structural" is experimentally shown if a bacterial cell is taken down to the absolute zero of temperature and then brought back and placed in nutrient medium. The cell is then able to grow as usual. Presumably the only thing preserved when absolute zero is reached (and absolute zero is a theoretical extrapolation; the best available data is 1.3° Kelvin) is structure. This indicates that the information necessary to specify that the object is a living cell has to be structural information since bringing it back to room temperature is just a matter of heating it up and disordering it.

Therefore, when we are called upon to de novo synthesize a cell, we really have to be just very clever organic chemists. The only thing we have to do is to get the structure right, and the system will start moving in the right direction all by itself. This represents a great simplification in the problem of de novo cell synthesis.

WOESE: Structural as opposed to what?

MOROWITZ: Information being stored by cyclic processes.

WOESE: Are there reactions going on in a test tube which is frozen down to absolute zero and then warmed up——

MOROWITZ: That means the information for those reactions is structural also. Let me give you an example. Suppose I were to store information clearly structural on a punch card. I then take the card down to absolute zero and bring it back and the information is still there. If I am storing information in a series of pulses that I am circulating through a reverberating network of some kind and if I take the system down to absolute zero and bring it back, the pulses are not there.

GARFINKEL: The easiest analogy is with certain computer memories. The very earliest ones, such as Univac 1, kept their memory in the form of sound waves in mercury tanks. If for some reason power is lost, then memory is lost. The more recent memories are magnetic cores that are magnetized a certain way; if power is lost, the same information is still in memory when power is restored. This case is really structural, whereas the other is a dynamic something that has to be kept going.

GRENELL: I would think that by freezing it you mean that you are interrupting certain loops that would be operative. When you warm it up again, it goes on.

MOROWITZ: In terms of de novo synthesis, this is rather important because it means that de novo synthesis is a problem of organic chemistry; it is not a problem of getting a system in the right structure and then pushing some of the atoms or molecules in the right direction. In terms of statistical mechanics we specify the desired q's and do not have to specify the p's; that is, we specify the coordinates and do not have to specify the moment.

Incidentally, this means that, in fact, we can calculate how many instructions, or how long a set of instructions, have to be given someone to de novo synthesize a cell in detail. From a number of arguments it is reasoned that it takes about 4 bits per atom to place it appropriately in a cell in a synthetic reaction. Thus, if we have a cell of 200 million atoms, this is 800 million bits, which is a reasonable message. We can get this in about a 1000-page book, provided coding is optimal.

This number represents both an overspecification and an underspecification. It is an overspecification in that we are specifying that every atom has to be the right atom in the right place in the cell, and we know a cell does not have to be specified in nearly that much detail. It is an underspecification in terms of the fact that we are assuming optimal coding, and organic chemists probably do not function by optimal coding.

POLLARD: Does this fit with the idea of the cell being under the instruction of a molecule?

MOROWITZ: No, the calculation is for the worst possible case. It is the case where the organic chemist has to put every atom in place.

POLLARD: It would have shrunk quite a bit if you had——

MOROWITZ: Yes. The second generalization I want to make is that there seems to be a ubiquitous and fairly restricted set of monomers and small molecules used in biological structure. Generally, these are the amino acids and nucleotides, but about 100 others which commonly occur can be added. However, from all of the vast class of organic compounds, this is a very small subset. Again, this seems to be one of the strong generalizations.

The third strong generalization I want to make is that wherever energy is used biologically, the last step along the process seems to involve phosphate bond hydrolysis, and the bond is usually ATP.

The fourth generalization is the one discussed earlier today, which is the seeming universality of membrane structure.

The fifth generalization seems to be the ubiquitousness of ribosomes in cells and the similarity of ribosomes in all cellular systems.

The generalizations become important because one question that has to be considered when discussing a minimal cell is the question, "What is the necessary apparatus?" It is very hard to define a living cell. I think this problem can be short circuited by a number of formal definitions.

It is fairly straightforward to begin by defining a self-replicating system. A self-replicating system is a system that can pick pieces out of an environment and assemble another structure similar to itself, so that both of the structures are now capable of repeating the initial process. Dr. McMullen gave an example of Dr. Penrose's little mechanical gadget (refs. 6 and 7) for doing this, which would be a simple case but would fit the general idea of self-replication.

There are numerous others. Homer Jacobson (ref. 31) published some in 1958 which were done with little trains on HO track—small, two-dimensional, self-replicating systems. Von Neumann (ref. 32) did it in a more complicated, formal way several years ago. But, in general, self-replication is going to include all these processes. It might include truly autocatalytic chemical reactions, if, in fact, they do exist. I think there is a real question as to whether autocatalytic reactions in this sense do exist.

The simplest kind of self-replication we can think of is that A + B goes to C. This is the general process we are looking for, where C is the completed unit, and A + B are the constituents in the environment. For this to be self-replicating, the process has to proceed by C + A going to complex I, complex I + B going to complex II, and complex II going to 2 C.

If we had a chemical reaction that really proceeded like this, it would certainly fit this definition of self-replication. In biology we take a second step and talk about autonomous self-replication. An autonomous self-replicating entity is one that can self-replicate in the absence of any other self-replicating entities. Therefore, when we discuss the minimal system, we shall not include obligate parasites simply because of the difficulty in sorting out the information between parasite and host. This is done for the purpose of discussion, and there is nothing fundamental here.

The next step is to introduce a biological autonomous self-replicating system, and here things become more difficult. One definition of an autonomous self-replicating system that operates at the molecular level is a biological autonomous self-replicating system. The things that are hard to exclude by this definition are crystallization processes. In the end, I think the reason for the difficulty of excluding crystallization processes by these definitions is that what is going on in the biological systems has features which are very close to crystallization.

We could define a biological system in this way, but it would have a bit of looseness to it; we would have to patch up the definition if we wanted explicitly to exclude crystals. It could be done in terms of growth, and so on.

ROBERTS: If you include the requirement that it has the capacity for evolution, I think you can exclude crystals and trivial things.

MOROWITZ: If we want to define a biological system that can fit all these other criteria at the molecular level and also has the possibility of evolving, which means that we are going to impose a certain minimum complexity on it, then we probably can exclude simple crystals.

POLLARD: And permitted to change in stable rate.

MOROWITZ: I assume that is what we mean by autonomous.

FORRO: How restrictive is the requirement that it come into part 2 C?

POLLARD: He is saying that this is exactly double. The 1.7 C is excluded.

FORRO: What I am trying to put into this is a requirement for spontaneous separation that is not in crystallization.

MOROWITZ: We can go one step further. We now have autonomous, biological, self-replicating entities; I think that if we are talking about exobiology we really must stop at this point. If we are talking about terrestial biology, I think we introduce what are conventional biological, autonomous, self-replicating entities; and we include here the additional feature that the subunits out of which they are made, the things that come out of the medium or the structures that you ultimately end up with, are the subsets of monomers and the small molecules that I talked about before.

Again, I do not think we have here defined life. I think we have run around the definition of life, but we have defined something that is useful because then we can begin to ask what a minimum unit would consist of. Therefore, this is, in a sense, the beginning of the problem at the theoretical level. It is only the beginning because we really have not done anything except introduce the vocabulary to talk about some of these problems.

At the experimental level, I think we do something quite different. We start out by asking, What is the simplest self-replicating entity? We can sharpen the question by asking, What is the smallest autonomous self-replicating, biological self-replicating entity? What is the smallest thing that can be found that will fit the criteria that I have given, that will take matter out of a medium in the absence of a host cell, and make two entities like itself? These entities must have the characteristics of normal biology; they must have the right monomer units.

WOESE: Before we go any further, let me state that in defining the minimal cell we must include the capacity to adapt, to mutate, in order to cope with the cell's environmental changes. Mutability must be one of the criteria for such a cell; its complexity has to be such that it can mutate by relatively small jumps.

MOROWITZ: That may come out of the minimal cell, but I am not sure we have to put it in.

WOESE: It would not live in any environment we can think of unless we——

MOROWITZ: The environment I am talking about is a flask in which I control the chemical composition and temperature and exclude any other cells to compete with.

WOESE: This is very artificial.

MOROWITZ: Of course it is artificial, but I am not talking about an evolutionary problem. I am trying to analyze what the process is.

WOESE: I am saying we cannot divorce it from an evolutional problem.

MOROWITZ: Conceptually, I can. All you are saying is that this thing I am going to think of may never survive in nature, that the idealized entity may never survive in nature. The one I am going to work with experimentally has survived.

WOESE: Your ideal minimal cell appears to be based upon a real cell, one which evolved under conditions of a changing environment, such as competition with other species. It is impossible at present to say that a particular feature of a cell—for example, protein synthesis apparatus—did not arise because it conferred a distinct advantage in the evolutionary process. Therefore, I do not see how we can extrapolate from a real cell to a minimal cell without considering evolution.

POLLARD: I think he has a point. If I understand what Dr. Woese is saying, that if we actually do search for these in the laboratory, if we search for them in nature, they will have to have——

MOROWITZ: They are going to have more criteria than I have given here. The only reason I have some slight doubts about the criteria mentioned is that I am not sure they do not come out of the criteria that I have set forth, particularly with the amendment that Dr. Roberts put in. They may be derivable from the criteria I have given; I just do not know.

LANGELAND: You ought to include adaptability in your criteria; then you exclude the crystallization.

MOROWITZ: Are you distinguishing between adaptability and mutability? Is this what you are saying?

LANGELAND: Yes, I remember you phrased it this way one time. We have a system like a cell, and we can give it several inputs. We may vary our inputs, but our result is two cells anyway; and that is a way of defining adaptability.

DANIELLI: Perhaps I misunderstood this, but it does not seem to me that we can exclude either capacity for change or adaptability from crystalline systems. Suppose we begin with a crystal that consists of a steroid, shall we say, or a fatty acid. We can progressively modify this by introducing components having, for example, some other polar group instead of a carboxyl group.

MOROWITZ: And we obtain cocrystallization.

DANIELLI: We have cocrystallization; and if we vary the environment of the crystal we will change the pattern of crystal growth so as to produce a differently shaped crystal. It seems to me there is quite a lot of adaptability and modifiability just in straight crystals.

MOROWITZ: But you are coming back to the problem of the paradox we had originally—that it is difficult to write the definition so that we unambiguously rule out crystals. I think the difficulty comes at this point because of the closeness between what we recognize as a living system and the many features of complex crystallization.

DANIELLI: Perhaps I could put in another point at this time. When we discuss this analogy between crystals and living material in the way we have begun to do, we are very close to the suggestion discussed this morning, that the cell structure will spontaneously arise from the components. I wonder whether, in fact, living systems do not differ from crystalline materials in that the components are so structured that the likelihood of organization arising spontaneously is very small. The advantage of this would be that then a mechanism would be necessary to induce a structure to arise; this would bring the whole thing under control—introduce the possibility of control.

If we rely on spontaneous aggregation, then the system is relatively difficult to control once we have some of the components assembled. For example, suppose that a cell needs to assemble certain components into two different units. How is it able to control the assembly of them into two different units in appropriate proportions if the structures form spontaneously anyhow? We need to have this system under control. This seems to be a reason for putting forward the entirely opposite hypothesis that spontaneous aggregation is to be avoided, and therefore the cell is likely to put in many components that will not spontaneously aggregate regularly. Secondly, the resemblance to crystallinity may, in fact, be misleading for just this reason, that a crystalline system is one that does spontaneously assemble.

BAUTZ: This is assembly but not division. Perhaps we can underline that.

POLLARD: It is an assembly, not a division.

MOROWITZ: We do have cases in biology, the TMV case discussed this morning, that clearly look like crystallization. Most DNA packaging, to come back to the point being discussed before, must involve a crystallization type of step. I think that taking membranes apart and reconstituting them, which is done, again looks like a kind of crystallization process.

BAUTZ: The TMV has first to multiply before it assembles.

MOROWITZ: Oh, yes. It is just that, finally, there is a step there resembling crystallization.

DANIELLI: Let us point out that forming TMV is something the cell does not want to do. This is a system that is out of control, as far as the cell is concerned.

GREEN: The fusion of broken membranes is more like the coalescence of oil droplets, and I would hardly consider that——

MOROWITZ: Except they seem to make two-dimensional sheath-like structures.

GREEN: I am not sure it can be explained that way. It seems to me that the hydrophobic bonds re-form, usually in these torn membrane systems, this would simply mean that the surfaces are of a similar character and have a tendency to refuse and exclude water. I would not classify such a process as crystallization.

MOROWITZ: Because it is a little less specific?

GREEN: Yes. With crystallization there is a tendency for like molecules to aline in a particular, regular fashion; but here we are dealing not necessarily with like molecules but with molecules sharing hydrophobic properties.

MOROWITZ: The similar feature is that you have a structured configuration because it represents the lowest free energy consistent with the constraints. I do not think I can rule out all Dr. Danielli's points; therefore, I may have to say that the process resembles crystallization. Let us look at the second part of the problem—the actual experimental search for the smallest systems. As noted, in the absence of being able to define simplicity, we look for the smallest systems. If we could define simplicity, we would really look for it explicitly.

Since we cannot define it, we look for the smallest system, with this idea in mind—that even by the time we are down to the conventional bacteria we have a relatively restricted number of atoms. Thus, if we can go to very small bacteria or cells that are appreciably smaller than bacteria, the number of atoms that we deal with is, itself, going to become very small.

Consider for example, the E. coli cells we talked about this morning. The cell volume is 2×10^{-12} cm^3. That means the nonaqueous portion has 0.5×10^{-12} gram; in molecular weight units this is 3×10^{11}. To put this in terms of the number of atoms, divide by 8, which is the average atomic weight of a biological atom; this becomes 3.75×10^{10} or 37 billion atoms in the E. coli cell.

This number is large but not frighteningly so. The point is, if a cell is one-tenth the linear dimensions of an E. coli cell, the number of atoms decreases by a factor of 10^3; thus we will be dealing with 3.75×10^7 or 37 million atoms. Thirty-seven million atoms is really a small number, as realized when we begin to put it in terms of some other terms such as monomer units per cell. There are about 20 atoms, on the average, to a monomer unit in biology. Thus, if we divide by 20, we have approximately 1.8×10^6 or 1.8 million monomer units.

On the average, it is going to take about 300 to 400 monomer units to make a polymer; we divide again by 300 and we get 6×10^3. We have only 6000 total macromolecules in a cell that is one-tenth the linear dimensions of the cell discussed this morning.

The idea that D'Arcy Thompson (ref. 33) stressed about the volume changing as the cube of linear dimensions becomes quite serious at this level. As the cell begins to decrease in size, the total amount of conceivable machinery becomes limited by the number of atoms. This is the idea I want to stress; this explains why the feeling behind this work was that, as we went down in the order of size, we went toward the level of simplicity. We cannot begin to worry about whether this cell has 100 000 different kinds of enzymes because it has only 5000 macromolecules total.

GREEN: That does not necessarily give the desired simplicity. If there is the possibility of making one complicated structure like a membrane, it is not much more difficult to make many such membranes.

MOROWITZ: It is not the total number; it is the total number of different kinds. Here the total number of different kinds is limited by the total number. You cannot have more than 6000 different kinds of enzymes because there are only 6000 molecules total. The number begins to limit variety.

GREEN: Yes, you are right.

MOROWITZ: Incidentally, the experimental work I am going to discuss represents the work of a number of people in three different laboratories; the Biophysics Department at Yale, the Department of Animal Diseases at the University of Connecticut, and the Department of Bacteriology at the University of Connecticut. I will not sort out whose work is whose because I want just briefly to describe what the cells are like. We began to look at small bacteria, and we examined Achromobacter parvulus and Veillonella parvulus; but in general, these cells turned out to be not as small as was initially reported. We also did some work with Dialister pneumosintes, which appears to be about 0.3×0.6 microns. The smallest bacterium has been reported by Van Itnersen and Robinow (ref. 34). It is a coccus 0.2 micron in diameter.

We began to investigate pleuropneumonia-like organisms, which were reported as being spherical bodies ranging in size down to about 0.12 micron in diameter. Most of the work we have done has been on three strains: one that I will refer to as A-5969; a second microplasmic laidlawii; and a third, H-39.

To describe briefly pleuropneumonia-like organisms, they are autonomous, self-replicating systems. In contrast to the bacteria, they lack a rigid cell wall. By the normal criteria formerly used for viruses, that of filterability, they are filterable. Most strains we work with, for instance, will filter through a 0.22-micron Millipore filter. Some viable cells will come through. With all the strains we get some cells through a 0.3-micron Millipore filter.

Most of our work was done on the A-5969. At first it was a sphere of about 0.3 micron in diameter. However, for some reasons not clear to me, it is now a sphere of about 0.6 micron in diameter.

KLEIN: How uniform are these things? Do they not have a tendency to coalesce?

MOROWITZ: This strain is quite good. In the electron microscope you see mostly a collection of spheres. When you begin to look at them in detail, you can see more structure than just the spheres; but this is not an enormously pleomorphic strain. There have been many reports of a number of these strains being extremely pleomorphic, possibly because they have no rigid cell membranes. Therefore, unless we are careful about how we look at them, much distortion can be caused. When we are careful with this strain, we generally find that the cells are approximately spherical.

GREEN: Does that mean each individual cell has a complete apparatus, or are there possibly, different cell types?

MOROWITZ: No, because we grow them as isolated colonies arising from a single cell. Experimentally we make a grid, Formvar-coated for electron microscopy, and put down a collection of cells. Initially, we see only single cells. The grid is then put down on a nutrient agar plate, and the nutrient diffuses up through the Formvar. An hour later the cells can be seen in the process of replication. The single cells give rise to microcolonies right on the Formvar, meaning that a single cell is an autonomous self-replicating unit. We use the term "clone-forming units" to indicate that it is an independent entity.

QUIMBY: Do the members of the clone all have this extremely small size, or are you dealing with a life cycle where one stage is small?

MOROWITZ: A population clusters pretty closely around the mean size.

These H-39 and M. laidlawii strains have been somewhat more pleomorphic than the A-5969, and we sometimes see cells as large as 1 or 1.2 micron. We see some smaller—as small as 0.15 micron. The H-39 strain is the smallest of all these strains we have worked with. It gives a titer through a 0.15 Millipore filter; in the electron microscope, and by the various other criteria, it appears extremely small.

DANIELLI: How complex is the medium?

MOROWITZ: Most of our growth experiments are done in a medium which is Difco's Bacto-Triptose. Difco will not tell us what they put in it, but it is probably a combination of enzymatic digests of beef and of yeast. It is a very complicated medium; and, in addition, a bovine serum lipoprotein also goes into the medium.

However, for the strain laidlawii we have a chemically defined medium. This strain was isolated originally as a saprophyte; the others were parasites. At the moment, I think we put 53 things into the medium. I do not think we will necessarily end up putting 53 things in the medium. But it is a long experiment to cut each one of them out, and this is being done more or less systematically.

The most complicated thing it needs is a peptide, or a mixture of peptides. The cleanest mixture of peptides that I can add to the medium with this strain so that it will still work is a

mixture of O-Tryp 8 and O-Tryp 10 from ribonuclease. This looks similar to Woolley's strepogenin story with respect to the peptide requirement (ref. 35). The rest of the medium consists of amino acids, vitamins, fatty acids, and nucleotides.

KLEIN: Any cholesterol?

MOROWITZ: All the other strains except this one seem to have a cholesterol requirement. This strain is not grown in cholesterol. We presently grow it with five fatty acids: myristic, palmitic, oleic, linoleic, and linolenic. It will grow, although very poorly, in the presence of oleic alone.

DANIELLI: That is important because the picture you are building up, it seems to me, is of a small organism that has shaken off the need for everything except the apparatus for assembling small units into macromolecules.

MOROWITZ: What you are saying is that it seems to have very little intermediary metabolism.

DANIELLI: Yes. And with a medium such as you mentioned, all the intermediary metabolism is effected outside the PPLO.

MOROWITZ: I have a suspicion, Dr. Danielli, that we will probably be able to cut out possibly 20 of these constituents. But I think the answer is partly "yes" in coming to a minimum system here. We are throwing away much intermediary metabolism and concentrating on the replication process.

PITTENDRIGH: Then why not go all the way to a virus?

MOROWITZ: Because I can still control this environment, and I cannot control the virus' environment, because the virus is growing inside a cell. The virus may be using information from the cell's DNA. This thing has to use only its own DNA.

DANIELLI: That is not quite true.

PITTENDRIGH: No, it uses the DNA of whatever organism made the peptide that was supplied.

MOROWITZ: But in the end the peptide requirement is nonspecific and can be coded in human DNA in the attempt to synthesize peptides. I cannot synthesize them, but Hofmann in Pittsburgh synthesizes these peptides (ref. 36).

POLLARD: I do not think there is a need to say there is any exclusion between studying viruses and studying these cells. I think they are somewhat different.

PITTENDRIGH: But I thought the point being discussed, or brought up, was an attempt to estimate the size and nature of the minimal, completely autonomous system—and this is not.

POLLARD: It is more autonomous than a virus.

MOROWITZ: The point is, any system is going to require an environment. We are just quibbling about the complexity of the environment.

PITTENDRIGH: But is not this the difficult theoretical point?

MOROWITZ: Let us put it this way. At least with this unit, I can sort out all the apparatus in studying it. If I study a virus, I have to include all the cell's apparatus; and I do not know which portion of it to exclude and which portion of it to study as part of my system.

DANIELLI: This might almost be defined as the simplest system in which DNA can multiply.

MOROWITZ: In the absence of another self-replicating system.

FORRO: We do not have to say that all the environmental information came from DNA unless we say via human DNA. If, as organic chemists, we were clever enough to synthesize these, the necessary ingredients would not have to be made by another organism. This could be closer to it.

MOROWITZ: All the things I put into this medium, in principle, du Pont can make from air, water, and coal.

DANIELLI: All the things that can be put into any medium.

MOROWITZ: Not for the E. coli. If du Pont could make the inside of an E. coli form air, water, and coal, we would not need this conference on de novo synthesis of a cell; thus there is a distinction.

ENGELBERG: It is merely a technological distinction that du Pont cannot do this. It seems to me this is all irrelevant.

MOROWITZ: I think there is a real distinction between an obligate parasite and a free-living cell. I think this is a clean biological distinction. The obligate parasite is using some apparatus within the cell; unless we know what that is, we cannot define the replicating system. If I have a free-living system in a medium, I can define the whole system.

WOESE: Would the people who are criticizing Dr. Morowitz's approach define themselves a little more clearly? What is being objected to? I see nothing conceptually wrong with his approach.

DANIELLI: We were trying to make sure (at least I was) of the criteria—the procedure for setting up. One thing that Dr. Morowitz pointed out was that if this unit he is describing could only multiply inside another cell, then he would not be able to distinguish what was the responsibility, as it were, of his unit and what was the responsibility of the cell in which it grew.

He is actually accepting as his criterion simply the unit capable of growing in a defined medium. He says he can define this medium and, therefore, it subscribes to his convention. The question would be, "Can you pare it down to something still smaller?"

Is that right, or do I misinterpret you?

MOROWITZ: Ultimately, yes. If I can find a smaller autonomous self-replicating system, I am willing to stop work on this one.

QUIMBY: Concerning your free-living organism on artificial medium, is that a very enriched medium?

MOROWITZ: No. I mean, the total nutrients are about 15 grams per liter—everything that goes into the medium—but this includes 5 grams of salt and 5 grams of tris.

QUIMBY: In other words, it is a fairly diluted artificial medium, but also complicated?

MOROWITZ: Yes. It is not unlike the tissue culture media.

QUIMBY: Have you tried taking about 15 or 20 of the constituents out of the medium?

MOROWITZ: All at once? No. What we are doing is taking them out one at a time. If I had insight concerning which 15 to take, I would do it that way.

DANIELLI: You could have your graduate students do it.

McMULLEN: You have no coconut milk in there, I assume.

MOROWITZ: No coconut milk; no horse serum. With the exception of the ribonuclease peptide, it is a synthetic medium, as distinguished between synthetic and defined medium.

ENGELBERG: If someone devised a means whereby viruses could be grown in a cell-free system to which certain enzymes and so on had been supplied, would this be consistent with your system?

MOROWITZ: I think it would be an autonomous self-replicating system, yes.

The next point I want to discuss, then, is, What are the constituents of these particular cells, and how much of the various things are in the cell? First let me say concerning the gross composition of the A-5969 that the cells are about 80 percent protein, 8 percent RNA, 4 percent DNA, and 10 percent lipid.

SZENT-GYORGYI: How much water?

MOROWITZ: This is in the nonaqueous portion. The dry weight amounts to about 25 percent of the total weight.

BRUCH: Is this your pathogenic strain?

MOROWITZ: Yes. These proteins have been looked at with respect to amino acid analysis. The nucleic acid base ratios have been determined, and the lipids separated on silicic acid chromatography. This has all been published, but I do want to note that these cells have about the same distribution of amino acid found in other cells. The DNA is double-stranded, the ratio of AT to GC is about 2:1. For the RNA, I forget the details, but it is well within the range that has been reported for other cells. This is true of the lipid composition, also.

In other words, in terms of this gross chemical composition—gross monomer composition, there does not seem to be anything unusual about these cells.

GREEN: Is it predominantly phospholipid?

MOROWITZ: Yes, except this 1-percent lipid is about 2 percent cholesterol, and the rest of it is mostly phospholipid.

We have tried to reduce all these figures to the quantity of this material there is in the smallest viable cell we can work with. This has been a problem we have battled, somewhat unsuccessfully, for the last 3 years. I think we know how to get around the problem now. But when we want to do chemistry, we have to work on a very large batch of cells; and, in general, under those conditions they may not all be viable or there may be a size distribution, and so forth. Indeed, there are problems of size distribution in most of the strains; thus we have difficulty in getting at the smallest viable units.

However, most of the attention has gone into getting the DNA per clone-forming unit. On the A-5969 strain I will give you an upper limit and a lower limit, and on the H-39 strain I will give an upper limit to what the DNA per clone-forming unit is. This is important because as the cell shrinks in size, the total DNA shrinks in size, so that the total coating capacity shrinks. This means, if we take coding seriously, that the total number of enzymes becomes limited and, therefore, the complexity of the system, at least judged in terms of the number of enzymatic steps it can carry out, becomes limited.

A-5969 seems to have a lower limit of about 40×10^6 and an upper limit of about 400×10^6, as far as DNA per clone-forming unit is concerned.

POLLARD: Is the problem the clone-forming unit or the DNA?

MOROWITZ: It is both. Also, there is a problem in this figure. The cells have become larger, and I am not sure that a clone-forming unit does not contain more than one genome at the moment.

LEIGH: Is this molecular weight?

MOROWITZ: Yes, molecular weight DNA. The H-39 strain has an upper limit of about 250 million at the moment.

First, looking at the upper limits, let us take the worst possible cases. If we are going to code proteins with this DNA, about the best we will do, making quite small proteins, is to code about 800 proteins from 400×10^6 molecular weight of DNA. That is about the outside limit of the number of different kinds of proteins. I would say the number of proteins we are going to be able to code in A-5969 is going to range somewhere between 40 and 800. For the H-39 I think we are going to have to place an upper limit of about 500.

This means that the total possible chemistry that this cell can carry out, assuming the enzymes are not multifunctional, is going to be limited to a fairly restricted number of steps. In principle, we should be able to draw the entire metabolic map for this cell. As will probably come out of Dr. Garfinkel's discussion, I guess we can now, in principle, go from the map to the computer, so that we have the hope of studying the cell metabolism directly by having the computer analyzing everything we do.

This is about all I will say now on how much of the various constituents there are. I regret the ambiguity in the number.

SLONIMSKI: Your calculation of the number of proteins is based on an obligate chain length, right?

MOROWITZ: Yes.

SLONIMSKI: If the proteins are polymers and they are made out of mixed chain lengths for different chains, the number of proteins may be infinitely greater.

MOROWITZ: Not infinitely, but considerably greater.

SLONIMSKI: Eight hundred in a multiple of——

MOROWITZ: Yes, if they can be dimerized at 640 000. Thus if it turns out to be a common phenomenon that we make peptide chains that can then be combined in a sensible way in a large number of configurations (it does not even have to be very large), it means that the total code length can be much smaller to get a considerable enzymatic diversity. Yes, that is true.

BAUTZ: If this is a common phenomenon, then we should get multiple mutations.

SLONIMSKI: I do not know whether we can do genetics on this type of thing.

POLLARD: Your DNA must be wrapped up rather tightly. How do you actually get that medium in? The point is, in the case of transformation somehow you have to hook onto the DNA, which is wrapped up in a very tight shape and capsulated. How can you possibly do that in a capsulated form? I would say this is a very bad cell from the point of view of the transformation process.

MOROWITZ: The knowledge of the rest of the "hardware" of the cell I want to describe comes largely from electron microscopy, and I will not go into the experimental details. Since this is a theoretical conference, I will draw the picture that emerges and give our interpretation of it.

KLEIN: Do not even draw it; describe it.

MOROWITZ: The cell is bounded by a double membrane. The evidence for this membrane is twofold: the electron microscope evidence and the dielectric dispersion measurements, which give the appropriate membrane capacitants obtained for the membrane of other cells. In addition, we have an area that appears to be a nuclear area; or, as judged from this morning's conversation, it may also be an artifact of fixation. We then see ribosomes.

POLLARD: Have these been precipitated?

MANILOFF: What do you mean, precipitated?

FORRO: How have they been fixed?

MANILOFF: Both glutaraldehyde and osmium tetroxide in the work Dr. Morowitz is presenting. Similar results have been obtained using freeze substitution.

MOROWITZ: This means that, when the artifact appears by several related methods, we believe it is something related closely to something that was there before we went through the process.

I did not have a chance to comment on electron microscopy this morning, but I do want to defend it. It is obvious that what is seen in an electron micrograph is a transform of what was in the cell before processing began. The restriction is that we have to be intelligent at each step about understanding what transformation is being made. Then we can obtain valid data from electron microscopy. I thought we were criticizing electron microscopy too much this morning. It is easy enough to sound impressive criticizing a technique, but it is much more important to use that technique to extract useful information about the system being studied.

POLLARD: If HDL is added to a silver nitrate, there will be a transform; and it will not make much sense.

MOROWITZ: If you understand what you are doing, it will make sense.

SAGER: Could we solve this problem by extracting ribosomes from the cells?

MOROWITZ: I have not reached the exciting part of the ribosome story yet. But, in answer to your question, what we have seen with respect to ribosomes has been seen in the cell and in centrifugal fractions from broken cells, including a purified ribosome fraction; the ribosomes have also been studied by ultracentrifugation. The ribosomal RNA has also been studied on a methylated albumin column. Everything I say about the ribosomes has been checked in multiple ways.

One curious feature of the ribosomes in these cells is that they are in aggregates. They occur in aggregates which, on inspection appear to be helical aggregates of ribosomes.

BAUTZ: Can you see the messenger being wrapped around?

MOROWITZ: The resolution is not that good. This is thin section. We would have to go about an order of magnitude or better on our electron microscopy, which conceivably can be done to see the messenger.

FORRO: I heard about 3 weeks ago that there are two rows.

MOROWITZ: The only other structures that appear presumably have to do with the replication of the cell. When we put these cells down on Formvar films, as I described before, we begin with a cell looking like a uniform sphere, except that it seems to have two light areas. In about 15 minutes a bleb appears at these light areas. We have measured the size of these blebs on a number of cells; it always appears to be the same size, as if it were a somewhat crystalline structure. The subsequent growth seems to take place from the bleb, leading to a binary fission; occasionally, however, the cell that comes off will be much smaller than the original cell and it will look like budding. Sometimes there will be more than two blebs emerging from a cell; thus it would not be straight binary fission but several subcells seem to be formed. In cross section, the bleb appears to be a structure something like an oblate ellipsoid.

FORRO: I am not sure about those lines. Before, you had very characteristic membrane lines; and now you have drawn fuzzy ones. Are those just thickenings?

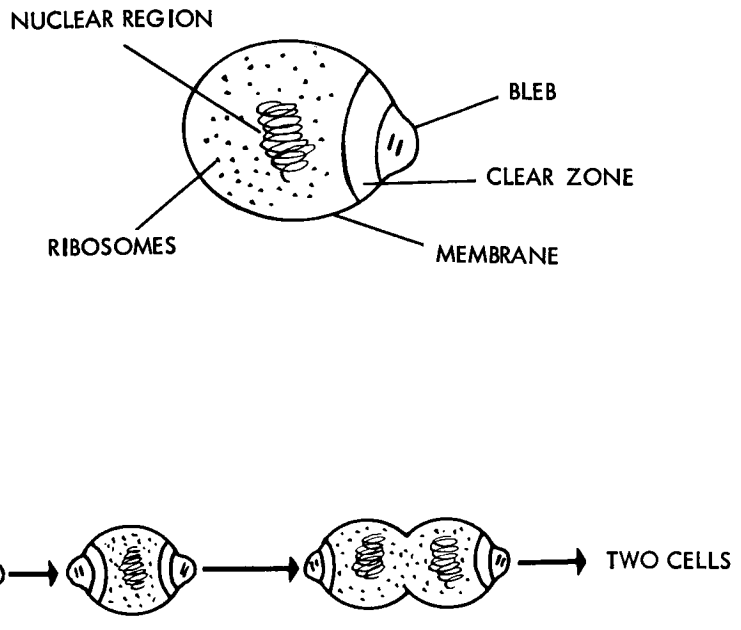

Figure 12. —Replication of A-5969. The upper figure shows cell morphology of the PPLO A-5969 (Mycoplasma gallisepticum) as revealed by the electron microscope. Organelles include cell membranes, bleb, nuclear region, and ribosomes. The lower figure shows cell replication, the life cycle of PPLO A-5969. The cell grows a second bleb at the opposite end from the original one. Nuclear replication takes place at approximately the same time, leading to two complete units which then pull apart.

DANIELLI: Are these double membranes?

MOROWITZ: I really think you ought to look at the picture (fig. 12) rather than take my interpretation.

GREEN: You do not see those double membrane structures in the interior but only on the periphery?

MOROWITZ: Right, and it seems to correspond with the bleb structure we see when we are growing them on film.

The total apparatus that we seem to have in this cell consists of the membrane, the ribosomes, and the central nuclear area here. In the dividing cells we seem to have the bleb. Of course, we have soluble proteins plus messenger RNA, transfer RNA and small molecules. But, as far as we can determine, this is about the total apparatus—the total cell hardware. Since this cell seems to function in a normally biochemical way, with the exception that its intermediary metabolism has to be supplied from the outside, it has appeared to us to be a good model system for the replication process. That is, anything that is absolutely necessary to the replication process must presumably be in this cell.

A corollary, which we do not expect to hold nearly as rigorously, is that a cell like this cannot afford much excess baggage because it is limited in the number of atoms; thus, it probably does not have much in it which is not an important part of the cellular replication process. At the moment that is the approximate state of the experimental minimal cell.

KLEIN: I am slightly confused. Do you have some ribosomes that are loose, that are disorganized, in addition to the ones you have shown?

MOROWITZ: Yes.

PITTENDRIGH: Is this an anaerobe?

MOROWITZ: It is an anaerobe but not an obligate anaerobe.

BRUCH: Is the particle that grows actively the same size as the particle that passes through the filter? You have not mentioned the life cycle of this organism, and I am still curious as to the role of the filterable particle in the life cycle.

MOROWITZ: Essentially, the life cycle is growth followed by binary fission.

BRUCH: But there is a range of sizes. The material passing through the filters could be a very minimal cell structure just with the coding information.

MOROWITZ: It has to have the coding information plus the apparatus to translate that coding information into functional information.

BRUCH: You mentioned this morning the range in cell sizes from 0.1 to 1.2 micron. What is that larger cell doing? Does it have more apparatus attached to it?

MOROWITZ: I think these cells have many control problems. Some of these strains have control problems with respect to size, not having a rigid cell wall. I think the details of the replication process will depend fairly critically on the environment, whether they are sitting on a surface, whether they are in a medium, and if so, what the surface tension is. In these cultures I think we are seeing a lot of noise of that type.

GREEN: Would it be possible to sort out a cell population by size, so that you have separated the very large and very small cells?

MOROWITZ: Yes.

ODUM: Do these cells in any way store fat?

MOROWITZ: I do not know. I gave the lipid content.

ODUM: That is presumably essential cellular lipid, but the cells might be larger if they had stored depot lipids.

GREEN: If you could sort them out according to size, could you then determine whether the larger ones, in fact, have more DNA or more of certain apparatus or hardware than do the small ones?

MOROWITZ: We have done considerable sorting according to size in these filtration experiments. Perhaps we can discuss these problems later. I would only tell you a long series of experimental woes as to the difficulties. I do not think they are inherent difficulties that cannot be licked so that we will not ultimately be able to get a population of small cells that are all viable and to pin down some of these numbers more definitively, more definitely. This, indeed, is what we are trying to do. It is just that we have not done it.

PITTENDRIGH: Is the error of metabolism biochemically known? Is there chemical evidence that the usual cytochrome system is involved?

MOROWITZ: There appear to be no cytochromes. The A-5969 strain is rather interesting in that regard. It metabolizes glucose down to pyruvate and lactate and then does not appear to use the pyruvate and lactate, at least in the medium in which it is growing, which may have a

large amount of exogenous competition. In the medium in which it is normally grown, the Embden Meyerhof scheme—where it appears to get its energy—seems to be completely dissociated from the synthetic scheme, which means that it makes a large amount of acid while it is growing. These other strains do not produce nearly as much acid.

HOFFMAN: No.

GREEN: There can be no Krebs cycle where there are no cytochromes. I do not know of any case. The Krebs cycle system of enzymes is an intrinsic part of the mitochondrial electron transfer system.

GRENNELL: Since this is a theoretical meeting, I wonder if we could ask what you think might happen if you took all this equivalent hardware, all the hardware you find in one of these, and shot it into a droplet of phospholipid and stuck it into your medium.

GREEN: You would hear a big cry.

MOROWITZ: I suppose my problem would then be convincing you that I did not have a contaminant after it started to grow.

I was going to say a word about what I think a theoretical limiting cell would have to be. It seems to me that a conventional cell will have to have a membrane; we are going to have to allocate about 100 angstrom units here. We must have at least one ribosome; there is no way out. One appears as a good lower limit.

ROBERTS: I would say zero. You would get the enzyme out of it, perhaps.

MOROWITZ: Except that we want to synthesize protein.

If we take a more conventional view, we will put one 70-S ribosome in there, which is about 150 angstrom units. We will have to put the 30 or so assembly enzymes in; that is, we have to include the activating enzymes and the polymerases. We will have to put in another 10 or so enzymes to take care of the energetics, the Embden-Meyerhof scheme. A minimum of about 40 protein molecules will be needed, and we will have to put in enough DNA to code all of these 40 proteins. This amounts to 40 million molecular weight of DNA.

KLEIN: Where did the membrane come from?

MOROWITZ: I am postulating that this is a necessary feature of the cell; if I take away the membrane, then everything will diffuse away.

KLEIN: There is lipid and protein in the normal membrane; thus there must be something to make it.

MOROWITZ: The protein is taken care of.

KLEIN: Oh, that is part of your assembly?

MOROWITZ: Right, and take five more enzymes for lipid metabolism.

DANIELLI: All those 45 proteins are the protein components, then, of the membrane; is that it?

MOROWITZ: I do not really care whether they explicitly get put into the membrane or not. This is the number you are going to need. You are going to need at least 45 molecules of protein.

GREEN: You could put some of the enzymes on the membrane.

MOROWITZ: You could. Do you mean to make them a structural part of the membrane?

GREEN: Attached to it, if you like.

MOROWITZ: In any case, this is what is going to give the structure. By the time we pack everything in and realize we need an aqueous environment for things to work, we are going to end up with a structure having a diameter of roughly 500 angstrom units.

ENGELBERG: Could we do without the membrane? If it were a gel, could we dispense with the membrane?

MOROWITZ: I started out by saying one of the great generalizations of biology was the existence of one type of membrane, and I want to stand by that for the design of this minimum cell. I want to make it minimal, but reasonably conventional, at this stage. For exobiology, you can be as unconventional as you like.

This structure will take in monomer units and assemble them into the polymers in such a way as to lead to the production of all the necessary parts of a second cell. Division is then necessary, about which we have said very little. From the point of view approached in studying very small cells, the unit just described would be the limiting small cell of this type.

What the actual smallest self-replicating entity we will find will be, I do not know. That has to be a continuing search, but it is an open-ended one. We do not know that we have the smallest one, unless we can show, theoretically, that the one we have is limiting.

DANIELLI: What kind of units are you assuming have to be assembled into the cell?

MOROWITZ: In this case, it would be amino acids, nucleotides, metal ions, and vitamins.

DANIELLI: In that case, that membrane will have to be full of permeases in order to get them in.

GREEN: That would be inherent in the conception of a membrane.

MOROWITZ: No. If I put specific permeases in—as Dr. Danielli is asking me to do—I have to start making this DNA terribly long, unless Dr. Slonimski's scheme enables me to have particular combinations make permeases for different things. In that case I can shorten the DNA.

I am going to be in trouble here. As the number of functional kinds of enzymes begins to go up, I have to expand this DNA, which means I have to expand the whole cell and make more membranes, and so forth. Therefore, I really have to get things in by permeases——

GREEN: I think you have to, in a way, and I think that is really the essence of Dr. Danielli's point. You have given detail to the replicating mechanism where you know about that detail. You have given no detail about the membrane system because that is the part about which we know less. As we know more about the membrane system, we will find that there are just as many parts, if not more, as you found for the replicating system.

POLLARD: It seems to me we can prove that permeases are necessary because we have to keep the thing neat. If we do not have permeases in they will not get out; and in this cell the tendency to get out is tremendous.

MOROWITZ: That in the end slows things down.

POLLARD: It might slow them to a stop.

LEVINS: Do you have any indication that these things are less efficient?

MOROWITZ: I do not know what would be a good criterion for efficiency.

SAGER: What is the doubling time?

MOROWITZ: It varies in different strains and the medium. It varies from about 100 minutes up to several hours.

GREEN: One point you have omitted is that there are many small molecules in the form of cofactors such as DPN.

MOROWITZ: I am assuming they are going to come in from the medium.

GREEN: I do not think you can assume anything like that.

MOROWITZ: If I am going to construct a minimal cell, you have to allow me to put things into the medium.

GREEN: In other words, you are not necessarily assigning to your hypothetical minimal cell all the attributes you have recognized in the small cell that you studied experimentally.

MOROWITZ: I think this is drawn somewhat on the basis of what you learn from there, but it is not a direct transformation.

GREEN: There you are certainly faced with the problem of synthesizing all these cofactors, and this synthesis would require special enzymatic apparatus.

MOROWITZ: What type of cofactors?

GREEN: For instance, if we have glycolysis, we would need DPN or TPN. If we have the replicating mechanism, it would require——

MOROWITZ: The things that we put in the medium here are quite extensive. For instance, Co-A is a requirement in the medium; and the way it gets in is somewhat troublesome.

GREEN: What about ATP?

MOROWITZ: I do not think so.

GREEN: Presumably it contains ATP.

MOROWITZ: I would be surprised if it did not.

GREEN: And we know that as part of protein synthesis we have GTP. Did you put GTP in the medium?

MOROWITZ: That we do not know for this cell. Remember, every time—I am not objecting to this—you force new enzymes on the system, you——

GREEN: I am not; nature is forcing them on.

MOROWITZ:——you put yourself into a problem with the total code length. In other words, you may be forced into the position of either a coding paradox or the necessity for the kind of multiple coding that is suggested. If the DNA fails by a large margin to provide all the functions you need, then you have to get those functions somehow. This kind of small cell may push you into a real paradox.

SLONIMSKI: May I go back to the PPLO cell? Let us suppose that it is actually the minimal experimental cell available. This, of course, is a cell that you do not synthesize but nature does. If nature synthesizes it, then it seems that it has an extremely strong selective advantage; otherwise it would not exist. Therefore, I propose to reverse the problem and look at the medium to see what it contains to cause this cell to have the selective advantage that it does in being such a minimum cell. What is in the medium in which PPLO lives to give this selectivity? Do you see my point?

MOROWITZ: One lives in the trachea of chickens.

SLONIMSKI: I would really spend much time in finding this out.

MOROWITZ: Another lives in sewage, and another lives in human genitals.

SLONIMSKI: I would abandon the last one. But you see, there is a very old story——

MOROWITZ: Excuse me, I have thought of this problem. The one that lives in the trachea of chickens is easier to think about. I thought that possibly this one could occupy an ecological niche that other cells could not get into because they were too big physically to get into the niche; that is, if there were porous places.

SLONIMSKI: I think it is astounding. I do not know whether it is original; but Darwin contended, apparently, that life is being created constantly in every pond, except that nobody can see most of it because natural selection wipes most of it out immediately. This minimal cell has not been wiped out; thus, if you know exactly what the environment is in the chicken trachea, I think you will have the clue for the minimal cell—not by looking inside the cell but by looking outside the cell into its environment.

MOROWITZ: There might be another environment in which you could get an even more minimal cell.

SLONIMSKI: Of course, but we do not know whether it exists, while in this case we know positively that it does.

MOROWITZ: However, if I am going to have to live with the chicken trachea, I might as well go back to the virus case. E. coli is an easier host than the chicken.

PITTENDRIGH: I hate to raise this question again, but I do not see why you do not. The only distinction is that here we have an obligate parasite whose conditions cannot be defined fully and reproduced fully. In the case of E. coli, we have an obligate parasite that can be reproduced in test tube conditions. For instance, there surely must be a spectrum of complexity all the way from the parasitic reproduction——

MOROWITZ: Let me give it to you at the most philosophical level.

POLLARD: I think there is a point here for people who think about viruses. Perhaps E. coli is a work bench for assembling. If, in point of fact, we cannot take nucleic acids, whirl them up, and put the proteins in place until we have the actual work bench to put it on, then we have something more than just the components. I think evidence exists that this is so.

MOROWITZ: Let me put this at the most philosophical level here. What I want to do is to begin with a flask containing a cell, a nutrient medium, and place it in contact with an infinite isothermal reservoir at temperature T. The cell will now give rise to two cells.

I would like to be able to predict this process—and this is a philosophical statement—from Schrödinger's equation or some other fundamental principle of physics. With that in mind as a kind of theoretical principle, I find it easier to approach the kind of systems I talk about here than if my system is a virus inside a cell.

In a sense, what you are saying is that a virus inside a cell is just as good a system to do this. There are two ways out. One, I can say this is the one I am interested in, not that one—that is the easy way out. My point is that I can define the interior of this flask much closer than I can define the interior of this coli.

WOESE: I think on the point of definition here we can draw a fairly clear line, as follows: We can supply the cells with any compounds, except linear polymers (for example, peptides, polynucleotides), of a defined sequence greater than a certain size. This size limit is determined by the probability of forming significant amounts of the particular sequence(s) in question under conditions of "random" polymerization; that is, where no "templates" are involved. For example, if we have to supply a molecule such as an s-RNA, the system is not autonomous; if we have to supply the tripeptide glutathione, the system is still autonomous.

MOROWITZ: You stopped at a tripeptide. I have to feed this a hexapeptide.

WOESE: That seems near the borderline; if the required hexapeptide must have a unique sequence, that is.

SAGER: There is an important problem here raised by Dr. Pittendrigh's question. By now we know a fair amount about the kind of genetic information carried in DNA and about the way it acts. It seems to me that one of the implicit questions being raised at this conference is whether, given that information in DNA or, let us say, in a virus, we have all of the basic genetic material necessary to make a cell. Is everything else just a work bench or machinery or something of that sort? Or is there an additional difference between a virus and a cell that is based upon the presence in cells of some additional components of an autonomous, self-replicating sort? If so, we can readily see that a cell is in as different a class from a virus as a living organism because it contains additional kinds of genetic information not present in a virus.

This, I think, is one way of stating the problem. There may be other ways of stating it, but they all involve the question of whether cells contain genetic information other than nucleic acid in composition. I have in mind as an example the possibility that two-dimensional templates exist carrying a special kind of genetic information concerned with the assembly of macromolecules into membranes and organelles.

MOROWITZ: Let me complicate this story further by what has appeared to me as a possibility for relating what is going on in these pleuropneumonia-like organisms or showing a relationship between some virus behavior and some cellular behavior. As you remember, I drew this picture with the bleb formation, which I said was a kind of crystalline structure. What I meant by "crystalline" was that it has a characteristic size and shape and then somehow a new cell seems to form around it. This new cell then has this crystalline bleb in it, which somehow breaks up and gives rise to two crystalline blebs.

DANIELLI: I want to raise a very strong objection to calling these crystals. "Organized," if you like, but not "crystals."

MOROWITZ: All right, two organized blebs. These, then, repeat this process. This is not unlike conceptualizing a virus that grows its own host cell. The fundamental characteristic of a virus is that it packages its DNA or RNA, and then it escapes and goes to a new host cell. There is a limiting case in the virus where it packages its material; and as it comes out of the cell, it pulls some cell membrane around it.

If we were to go one step farther where the virus pulls a larger hunk of the cell and within its DNA had the information to carry out polymer synthesis, then we would have a case with features very much in common with the virus growth except that the virus is carrying its own host cell along with it.

Perhaps it is possible to envision many cells in that regard so that there is a continuous transition here between the possibilities inherent in these kinds of growth pattern. As I said, we can go one step further so that the DNA carrying the code with it has enough other apparatus and the information to carry on further macromolecular synthesis. I am not saying it is the same. I am saying we could think of a continuous gradation of this kind of behavior.

SAGER: What are those blebs? (fig. 12.)

QUIMBY: I was going to ask the same thing. Have you followed the blebs in a kind of lapsed time photography, or anything like that? Do the daughter cells each contain a bleb?

MOROWITZ: Dr. Quimby, when you talk about lapsed time photography, you must remember we have to look at these under the electron microscope. Under the optical microscope, the phase

microscope, we see a dot. The cell is dead when it is under the electron microscope; thus time lapsing is very difficult. We try to do the equivalent; we try to phase the culture and then select time samples.

QUIMBY: The equivalent, of course, is what I had in mind; the fact that you have two of them, one on each side of the cell, simply looks like the beginning of replication.

MOROWITZ: That is what we believe, too.

KLEIN: To come back to this point about the degree of autonomy in your survey of small cells have you looked at blue-green algae? It seems to me some of these get extremely small. It would seem that here we have a cell which is not only photosynthetic but has a tremendous biosynthetic capability. Some strains, for example, fix nitrogen in addition to carbon dioxide.

MOROWITZ: I wonder if we could defer that until after Dr. Starr speaks; it will have more relevance then.

STARR: I have only a few remarks to make. I could begin perhaps by attempting to answer Dr. Klein's last question. He asked about the lower limit of size of blue-green algae. They are self-nourishing and, like self-nourishing bacteria, the lower limit of size is about 1/2 micron. I do not know of any micro-organism smaller than, let us say, 1/2-micron sphere or a rod of 1/2 micron by 1 micron, which is able to grow essentially with no more starting material than ammonia and acetate or ammonia and glucose, for example. As a matter of fact, listening to this discussion, I think we must consider that fact as a starting point—that an organism having all the machinery for making everything from ammonia and glucose apparently experimentally through evolution has ended up being about 1/2 micron in diameter. It must take that much space to pack everything in.

KLEIN: This one is that large also.

STARR: Yes. I say this by way of beginning with the most common bacterium or blue-green alga, one which is capable of synthesizing everything needed to make more cells from very simple starting materials. One thing came to my mind in listening to Dr. Morowitz, and that is that all organisms smaller than that are no longer self-nourishing. Almost every case, at least the well-authenticated ones, are approaching parasitism if they have not reached rank parasitism. As an extreme case in that direction, I would like to take a moment or two to describe our laboratory pet at the University of California at Davis.

Last year the man fated to be my colleague this year, Dr. Heinz Stolp of Berlin, was working on phage ecology, making a comprehensive search for the bacteriaphages of Pseudomonas. As one usually does in looking for new phages, he observed in some plates not thrown away at the end of the day some slow developing plaques. After three or four days, these began to look like just ordinary phage plaques on lawns of the Pseudomonas that he was then studying.

To make a very long story short, with the type of intuition of the prepared mind and the fact that he is simply a bug hunter at heart, Dr. Stolp looked at those plaques by taking a bit and putting it on a phase microscope, and the thing was just crawling with exceedingly tiny bacteria. He is now working with me, and we have explored this little creature for the past year. It has astonishing properties in addition to its tiny size. The size, the parameter which interests us here, is on the order of 1/4 micron in diameter. They range up to as much as 2 microns in length. A typical one would be 1/4 or 3/10 micron in diameter and 1 micron long. The volume relationship compared, let us say, to E. coli would be roughly 1/50, possibly 1/100, of the volume of the ordinary bacteria.

The size is not the astonishing feature, however, and it only came to my attention as the result of following Dr. Morowitz's work. This is not the feature that attracted me or

Dr. Stolp, who, indeed, is the discoverer and deserves essentially all the credit for whatever these remarks are worth.

This organism is obligately predatory, ectoparasitic, and makes its living by lysing susceptible bacteria that it has previously attacked physically, attached to momentarily, broken asunder, and then proceeded to live on the juice. It is a strict parasite. It cannot be grown even on faintly killed cells—that is, slightly pasteurized ones. It will not grow even on cells that I have broken up by every armament of the biochemist. Any device that breaks cells already destroys the utility of the juices for the growth of this little creature. We have now studied it extensively in terms of its habitat; it occurs in every gram of soil we have ever looked at to the extent of 10^{100} individuals and in every milliliter of sewage to the extent of 100^{1000} individuals. It is all over the place.

We have now found, using as propagating strains with ordinary double-layer technique that a phage expert would use, some 20 or 30 different clones in terms of the propagating host. These all turn out to be one kind of organism—a tiny, slightly curved rod 1/4 to 3/10 micron across, 1 micron long, and having a most unusual flagellum as the other amazing feature of the organism. The flagellum is several times as long as the bacterium and, most importantly for the way it earns its living, that flagellum is 50 millimicrons in diameter (a typical flagellum is 5 millimicrons).

This flagellum seems to be ordinary bacterial flagellum as far as we can tell by fine structure. It propels that bacterium roughly 10 times as fast as any other bacteria move. We have various estimates, comparing with the literature. The flagellum moves quickly, as can be seen just by looking at it. When it hits a bacterium, it really does so. Despite the small mass of this little creature, it will literally knock an E. coli cell completely across the field, and computations can be made from that fact to indicate the speed it has. It hits E. coli broadside and remains attached for a moment. The bacterial cell, which is so attached and attacked, becomes a sphere—"spheroplast" may be a proper term for it, but we are not sure yet—and within 30 or 60 seconds is completely broken and falls to pieces. There is nothing left of the bacterium. This organism is otherwise an ordinary bacterium.

POLLARD: Hydrodynamics of collision is purely a physical term. This is a problem for theoretical biology.

STARR: It is an ordinary bacterium. It has a cell wall, a double membrane, and a much invaginated membrane structure; intracellular invaginations go all through the place from the very small amount of thin section.

To come back to Dr. Morowitz' main theme, here is a small bacterium with extra baggage, though with conspicuous consumption. It is only 3/10 micron across; yet it has a typical membrane, a typical cell wall, and the extra baggage, the flagellum, and yet, it packs all the information needed. Admittedly, though, Dr. Green, it does not have everything that would be needed if it were a self-nourishing organism.

Having built up what sounds like a type of theorem that a very tiny organism must necessarily be fully parasitic because it does not have space for all the machinery, I now must demolish that with the unfortunate observation that from these parasites we have been able now to select three living saprophitic derivatives that, in turn, could be turned back to the parasitic form by selection. These three living saprophites are also very narrow: however, they are not uncomplicated in their nutritional requirements and are perhaps as complicated as microplasm. We have never really grown them in any defined medium other than the usual mixture put together in the laboratory to grow this kind of organism. The little creature is called Bdellovibrio bacteriovorus, a name suggested by Buchanan who saw some morphologic and functional relationship to leaches. "bdello" means leach.

POLLARD: Does anyone want to argue with this remarkable phenomenon?

PITTENDRIGH: Is it an anaerobe?

STARR: No, the host bacteria are all anaerobic, and these are growing as plaques. In other words, the colony of Bdellovibrio is a plaque on E. coli or Pseudomonas lawn. They are remarkable in one capacity; they are all catalase negative, as are all of the parasites and the saprophitic derivatives. I would say from the very scanty growth the saprophites make that they are just barely existing. We have not really studied what is needed to make them grow better. They reach enormous populations. The parasite, for example, will knock down a coli culture which has 10^9 cells/ml and become 10^{10} Bdellovibrio in a period of a few hours.

LEVINS: It occurred to me that some of the problems relating to the evolution of cellular structure are similar to the problems we are working on in the evolution of ecological communities and that the same techniques could be used. I am interested in the problem of the direction of natural selection on structure. This morning we had some discussion about the role of structural differentiation within the cell, primarily in terms of diffusion. The selective advantage to setting up a structure that brings enzymes together is the reduction of the diffusion time from surface to surface. The selective advantage should be roughly proportional to the number of enzymatic steps brought together, and should also increase with the size of the cell since the average diffusion distances would increase. Thus there would be an ascending curve of selective advantage for a structure against cell size.

On the other hand, the cost to the organism of adding a new structure decreases slowly with cell size. In a very small cell, the addition of a structure may duplicate the amount of DNA needed or the quantity of protein; whereas in a large cell the proportional increase is less. Thus the advantage and cost curves will eventually cross. This means that the addition of new structures will be advantageous beyond a certain size but less so, or disadvantageous below that size.

There is a second approach to the evolution of cellular complexity and spatial differentiation. Suppose that we have two enzymatic processes, both vital for survival, with different pH, redox, or other optima. Imagine that the two axes of a graph represent the efficiencies of the two processes, so that each point represents the efficiencies of both for a given pH or other internal state. The set of all points representing these efficiencies will be a closed region of the plane. A mixture of inner states (spatial or temporal) will be represented by a point on the straight line joining the points of the states separately. In this way the original set of points is concave; a mixed condition in the cell gives some efficiency points that cannot be attained by single states. Since the overall survival value of the cell will be some monotonic function of the values of each process separately, the optimum state is that which corresponds to the point maximizing this function over all states or combinations of states.

Given this preliminary differentiation of the cell spatially, we can now look at another process while thinking of the same process taking place in both parts of the cell. A number of different enzymes carry out the same process. Now the axes represent compartment 1 and compartment 2 of the cell, and each point represents the efficiency of an enzyme in both of them. In that case, we can then have a set of points of the same kind and ask the question, "Is it better to have a single enzyme that is carrying out the process optimally in one of the compartments or a set of enzymes with properties that do intermediately well in each?" Again, the same kind of argument will work; if the set is concave, the optimum situation will be one in which we have a mixture of enzymes or a single enzyme that does well in one part of the cell but not in the other.

If that is the case, the complexity is compounded now when we have even greater lack of homogeneity, dishomogeneity within the cell, and thus we have an evolutionary push in the direction of greater structural complexity.

DANIELLI: Is this where isoenzymes come in?

POLLARD: He came at me rather fast when he discussed the diagram. The first one I understand, but the second one lost me in terms of the concavity and the convexity. Am I the only one who is lost?

LEVINS: The concavity and convexity come about if the two peaks for the optima of the two processes are far apart with respect to the inflection points of the curve. In other words, if the curves are narrow, if the requirements are very specific compared to the difference between their optima, we obtain a concave relationship; otherwise, we have a convex one.

POLLARD: How long does it take for this kind of evolutionary process to work? How many divisions are there? What is the mechanism? We see the advantage, but what is the rate?

LEVINS: To do that, we first have to put in the measure of heritability and, secondly, a measure of the adaptive advantage which can be calculated if we consider the efficiency-against-environment factor curve for a pair of enzymes.

POLLARD: Let us say it is going to happen and we are going to observe this thing actually showing in, for example, 100 generations; have you any figures?

LEVINS: I have no idea how long it would take for the genetic variant to arise to be selected.

POLLARD: Could you not invert the argument by saying you know it happens in 100 generations? Could you then say what the genetic variants would have to be?

LEVINS: The rate of change depends on the selective advantage and also the genetic variability available. I do not think we know either of them yet; but, in principle, I think they are measurable.

POLLARD: If I give you the time and the number of divisions, could you give me the variability?

LEVINS: I could give you an expression that combines the genetic variability and the selective advantage; then, calculating the selective advantage separately, I think we can get back to the genetic variability.

POLLARD: There are a number of points really should be touched on. Some of these do concern membrane. I wonder if for a few minutes anyone who has any remarks about membrane would make them.

STARR: I would like to make another remark on behalf of some interesting work being done in our department, though not in my laboratory, by Stanley Holt, a student of Gerry Marr. Stanley Holt, looking at Spirillum roseum fine structure as a function of the light intensity under which the organism is cultivated, has been turning up an interesting relationship in his electron micrographs. Under very low light intensities, there is a most extensive intracellular membrane system. It is an extreme. There is a great amount of intrusion of the membrane. At very high light intensities, the opposite is seen. In fact, he sees essentially no intracytoplastic membrane intrusions. The relevant data are available for your examination if you would like. They are in the form of thin sections of the Spirillum grown at both high and low light intensities.

GREEN: Is the extension of the membrane in the interior an extension of both layers or of only the inner layer?

STARR: Both, as far as I can determine, although I must admit my lack of competence in this area. The membrane was simply sent along in line with what I thought the discussion of the minimal cell would be; namely, what structures can be lost and still give a functional cell. Accordingly I prepared as best I could a long list of such characteristics, this being one. I do not know much about the details of membrane structure, and I am sure there are people in the room who would be able to interpret these.

SZENT-GYORGYI: Dr. Morowitz, your minimum cell is really very complex, it could not arise continuously; thus, concerning the origin of life, this is a blind alley, so to speak.

MOROWITZ: Yes, I think this is essentially true because what is talked about as a minimum cell here is a minimum cell using the present kind of hardware that has been evolved. These structures must represent a great deal of evolution from primitive cells.

BAUTZ: Since I like to think of DNA as an evolutionary consequence of RNA, a very primitive cell could do with RNA alone; I mean there is no a priori reason why we must have both DNA and RNA.

McMULLEN: But RNA would be almost as complicated and just as unlikely to be spontaneously formed as DNA. In other words, what does this definition "de novo" mean? This is something I wanted to ask, myself, and thank you for bringing it up—the distinction between de novo synthesis, biogenesis, and biosynthesis. At some stage I would like to hear the conference discuss biogenesis—theories of biogenesis. I realize that the practical confirmation of this is questionable, but I noticed in the original program we had an item concerning the prehistory formation of macromolecules. I wondered what had happened to it. Are we leaving this to the theologians?

POLLARD: Not exactly the theologians. Dr. Fox is coming in to talk about it. At least he is coming on Sunday. In my mind the de novo part is very simple. It is something you can do yourself. Somebody actually does it in the laboratory and that is de novo. If it is biogenesis and it has to go through many processes that we cannot simulate in any way for various reasons then it can be biogenesis, but de novo means we can do it.

McMULLEN: But are we going to discuss biogenesis on Sunday? Very appropriate!

POLLARD: With Dr. Fox here, it will be inescapable. The objective of the conference was for us to go home and make cells after we had finished. We do not want to go back too many years in the process to do that.

McMULLEN: Do you think you can make them without considering the evolutionary aspect?

POLLARD: This is what the conference is going to decide for us.

McMULLEN: Is it really going to decide it?

POLLARD: Yes. Because I do not believe we can continue much longer and make any sense.

D. ORGANELLE ASPECTS

1. Specificity of Organelles

Discussion leader: M. Ycas

DANIELLI: I would like to ask Dr. Slonimski to begin or perhaps Dr. Ycas will start. But first I want to list a few points I have noticed as we proceed in this area. First of all, one thing I think would be really valuable to have in connection with the study of organelles would be the theory of the physical state of these two-dimensional membranes.

We are sure that in these systems we probably have several phases coexistent at any one time. Transition between these phases is important. We are just beginning to make bimolecular membranes with defined structures—just beginning is exactly the word. I think we already know enough to put up a crude model of such a membrane as a basis for theoretical studies.

Another point I would like to comment on is the one that has been written up about the static capacity of such membranes. I think that the small variation in static capacity which we observe reflects the fact that capacitance is an insensitive measure of the variations in function in such membranes. For example, we can probably have a variation of ten thousand-fold in the electrical conductance of such systems with a very small change in the static capacity. And I would not expect, for example, the static capacity to change at all significantly if we poison, say, the special glucose transport system existing in such membranes. Therefore, I think what is being measured there is invariant properties of the membrane, which simply represents the fact that we have continuity of the membrane as such.

There is another point relative to some of the earlier discussion. The question was raised of what a nuisance it was to provide the extra DNA to provide permeases for these membranes. However, I think the fact has to be faced that a membrane that was just a bimolecular lipid structure would probably be no use to a cell whatever. In the original papers I wrote on this subject, I defined this as a structure correct to a first approximation and postulated enzyme-like areas in this membrane, which had the function of securing the transport of particular species of molecules. I have only come to appreciate recently that there must be another type of special structure—the receptors which are not necessarily concerned with the transmission of molecules across the membrane, but which are concerned with the transmission of the effects of the presence of a molecule on one side to the far side of the membrane.

I think the situation which we have to consider, therefore, is that every membrane-based organelle, at least, consists of a basic lipid structure for which we could, I think without too much difficulty, work out physical theory. The special functions of the membrane and, as a matter of fact, those functions which make it a biological membrane, depend upon the receptors and the permeases that are present there. When we discuss the specificity of these membranes, it is really the permeases and receptors with which we are concerned and only secondarily, I think, with the characteristics of the lipid phases. (I could be wrong about this).

At this point, Dr. Ycas, I would like to turn over the leadership of the discussion to you.

YCAS: Dr. Slonimski will now consider some of the data obtained by study of yeast mitochondria. His work is basic to the problem of the existence of membrane inheritance, and I am sure it will be of great interest to all of us.

SLONIMSKI: My comments are purely experimental and not theoretical. I do not consider myself a theoretical biologist; as a biologist, I am a rather pure biologist.

I would like to discuss first the definition of the mitochondrion. Everybody knows what a mitochondrion is from a classical picture, but I think the proper way to ask what a mitochondrion is is to withdraw various factors from the mitochondrion and ask whether, according to our definition, it still remains a mitochondrion by mutual consent. We can withdraw all the cytochromes. The large part of the mass, the protein mass of the mitochondrion, is composed of cytochromes a, b, and c—the dehydrogenated enzymes; and the Kreb's cycle enzymes. Either by mutation or by repression and induction phenomenon, we can withdraw, for example, cytochromes a and b, the activity of the corresponding enzymes, and several dehydrogenases; morphologically it still looks like a perfectly good mitochondrion. We can also withdraw cytochrome c. We have a mutation that prevents the formation of cytochrome c; nevertheless, it looks perfectly like a mitochondrion and still catalyzes respiration.

Concerning the membrane the question becomes much more operational. If yeast is grown under strictly anaerobic conditions, and if steroids are supplied in sufficient quantities, the yeast will grow. When we make serial sections of this yeast, we do not find anything that looks like a mitochondrion in the sense of the definition of the classical membrane having spacing. Some microscopists do see some kind of a vesicle; others do not. Some say that

there is one vesicle per cell; others say that there is one vesicle per three cells. I do not know, but there is certainly no mitochondrion by the morphological criteria. From this point of view all the electron microscopists would agree that what they do see is not a good mitochondrion.

Nevertheless, within about 40 to 50 minutes after exposure to oxygen, we can see all the mitochondria formed by all the criteria: morphological, spectroscopical, enzymological, and immunological. Thus, either the time module of the membrane has been preexisting or it was completely formed de novo. If it has been completely formed de novo, the cytoplastic mutants show 100-percent irreversibility. To my knowledge, this is the only mutant in the whole field of microbial genetics that has been tested for a reversion rate of 10^{-12} with a reconstruction experiment. All the mutants having exactly the same phenotype to revert, and with normal frequency. Here we are faced with the problem of complete hereditary loss of function without a concomitant loss of morphological structure. On the other hand, we have temporarily phenotypic reversible loss of the complete morphological structure without the hereditary loss of the function.

As Dr. Ycas said, there are agents that induce a loss of the mitochondrial continuity. The classical example is the work on acriflavine. In acriflavine the mother cell retains good mitochondria for five or six divisions except for the daughter cells which are irreversible cytoplastic mutant. For purely technical reasons we do not know what happens to the mother cell after the fifth or sixth division. These experiments have been done by micromanipulation in which the buds have been detached from the mother cell. When there are 32 or 64 cells, it becomes technically difficult to keep track of the mother cell and to know whether it has been transformed into the petite or whether it has simply died. Therefore, after many divisions there may be a transformation of the wild type into a petite, but for at least six divisions there is none. This picture, of course, is mostly compatible with the idea of continuity of the membrane. Let us say the mitochondrial membrane does not go through but is somehow clogged or aggregated; it does not go to the bud when acriflavine is present. Or, let us say that in any kind of replicating system involving nucleic acid, whether DNA or RNA, it does not make any copies that can be transferred into the progeny but they are still present in the mother cell.

Recently we have discovered another agent, 5 fluorouracil, that does the same thing. It introduces 100-percent mutation during cell division only, but with the difference that it induces it simultaneously in the mother and in the daughter. The experiment is simple—5 fluorouracil is put through a synchronous culture of yeast cells and buds appear. This is specific for 5 fluorouracil; 5 fluorodeoxythiamine does not do it. Reversal experiments with uracil and thiamine and all possible combinations suggest that ribonucleic acid is involved. The nucleic acid component favors this type of interpretation. Whether it is an episome, a chromosome, or a specialized ribosome does not matter.

If we want to correlate the membrane continuity and the nucleic acid component in the continuity of the mitochondrion, we can speculate that there is a system analogous to the whole replication system of bacteria—analogous except that it is much smaller and strictly within the cell. The replication system is composed of DNA and a piece of membrane, and there is a mutual interdependence between the membrane and the memory, which is the cell wall of the bacterium, and DNA, which is the bacterial chromosome.

We can speculate (and here it is easy to speculate because the experimental evidence is hard to obtain) that the mitochondrion continuity is due to the ribonucleic acid component and a piece of the membrane. Either the membrane permits the replication of the ribonucleic acid component or, on the contrary, the ribonucleic acid component permits the membrane to grow. Whatever the block is, either one side or the other, the system becomes irreversibly changed.

The second remark I would like to make, concerns the petite. It is also purely experimental and concerns the function of the mitochondrion. The function of the mitochondrion is to produce energy for oxidation and phosphorylation coupled to it. It is measured very easily by the rate of respiration. Most of us who have measured respiration are accustomed to a given value of the respiration. For example, yeast cell respires with a Q_{O_2} somewhere between 0 and 100. I do not remember the value for the liver, but I think it should be around 20, as it is for the kidney also.

I recently came upon a system where the same yeast cell can respire with a value of about 2000, or perhaps even 4000. It happens in this way: If I take a cytoplastic mutant that has permanently changed mitochondria, does not respire, has no cytochrome a, no cytochrome b, and no cytochrome oxidase, its Q_{O_2} is 0. It is really smaller than 0.3. This is my limit. I take a second mutant, which is a genic mutant. It is not a cytoplastic one but is phenotypically exactly alike. It does not respire; it has no cytochromes and no enzymatic activity. If I cross them, they do complement. This means that the cytoplasmic mutant has something that the genic did not have, and the genic has something that the cytoplasmic did not have. Therefore, the diploid issued from the cross of these two haploids does respire.

The strange thing is that the zygote respires at a tremendously high value immediately after fusion; but within an hour, or an hour and a half, it becomes less prone to respire and eventually returns to its normal respiratory value. This means that there is a tremendous overshoot in the kinetics of the two—from a value of zero to an overshoot and then to stabilization to the normal value of respiration. This overshoot is so fantastic that the mitochondrion, which we consider as a perfect machine for respiration (when we have the value of 100), simply can do 20 times more. Now, what the actual system of regulation is I do not know. This requires isolation of zygotes in larger amounts and this is technically difficult.

PITTENDRIGH: If you withhold oxygen from the zygote for a while and put it into an anaerobic state, will it return to the same state?

SLONIMSKI: That is difficult since they reproduce very poorly in the absence of oxygen. These experiments are technically difficult because we must have pure zygotes.

PITTENDRIGH: What I am really after is this: Once it has returned to its plateau, if it is put back into an anaerobic state again——

SLONIMSKI: They behave exactly as if they were completely normal diploids. There is no overshoot.

PITTENDRIGH: You do not get a transition?

SLONIMSKI: No. Everything we do from here on is perfectly normal. But if we go to no oxygen, we have no respiration; if we go back to oxygen, we do not have any, either. This overshoot is, of course, interesting in several ways because it is regulated by many different genes. We have just one cytoplasmic mutant, but we have several genic mutants; phenotypically they look very much alike, but they are genetically located on different parts of the chromosome.

GREEN: Has not only the oxygen uptake but also the oxidative phosphorylation ever been determined?

SLONIMSKI: In zygotes? No, we do not get enough zygotes to manipulate.

GREEN: An interesting phenomenon which everyone is very aware of from studies of mitochondria is that mitochondria, as normally prepared, operate at extremely low activity from the standpoint of respiration and, if damaged so that the oxidation is no longer coupled to the synthesis of ATP, the rate may be increased by a considerable factor. This increased rate is usually the indicator that the system has been uncoupled. It is conceivable that in the initial

phase there is a transition period when the mitochondria are really uncoupled and, therefore, they have this extremely high rate and, with time, the mitochondria gradually undergo repair. It is actually possible to repair a damaged mitochondrion by appropriate means. Then, once recoupled, it will resume its normal, comfortable rate of respiration.

SLONIMSKI: This is quite possible. It has been tested experimentally, not by measuring oxidative phosphorylation directly. By indirect measurements we know the value of the uncoupled respiration by uncoupled genomes in yeast, and it goes from 100 to 170. This is still a tenfold overshoot and the uncoupling in animal mitochondria, which is tenfold as you say, goes, for example, from a Q_{O_2} of 10 to something like 100.

QUIMBY: One hundred what?

SLONIMSKI: Microliters per hour per milligram of dry weight.

The last point I want to make concerns cytochrome c, which is the only protein we can do something with in terms of primary protein structure. Its complete sequence is known, and I think it is the only protein for which complete amino acid structure is known for two or three or four species, beginning with yeast and ending with the human heart.

For the other cytochromes, the situation is much more difficult; they are much more complicated, and the amino acid sequences are not known. Thus, philogenetically speaking, cytochrome c is always 100, 140, 180—it does not matter. We know where the heme is, and we know about the pieces of the amino acid sequences that did not change between yeast, wheat and human heart. There is a piece for about 10 or 15 residues that did not change. Another part changes completely. The c terminal, however, is the same. (This is not my work but that of several independent biologists.)

Three years ago we found a mutant in yeast that had little cytochrome c. We took the cytochrome c (segregated a perfectly good single Mendelian gene that had been localized), isolated it, and did the amino acid analyses. To our surprise we found that for a single yeast mutation we obtained at least four and probably six substitutions.

CROW: Are these adjacent substitutions or scattered?

SLONIMSKI: Perhaps they are all adjacent; this would help us. Then we did pieces and found that at least three of them were nonadjacent. Surprisingly there was an amino acid substitution within the piece that did not change at all during evolution.

We were greatly bothered because such change in amino acid sequence as the result of gene mutation was unexpected. Then, we looked more closely at the system and found an explanation that makes the system more orthodox. The normal mitochondrion has two cytochrome c's that are identical in function and in spectrum, except for this amino acid substitution. Normally in a wild type of strain the proportions are 99 to 1 and even 99.7 to 0.3; thus the minor component is not seen.

We also looked for other mutants, and we think we have a third one. However, for this we do not have amino acid data which are sufficiently demonstrative, but let us talk only about those two. We call them iso-1 and iso-2.

Personally, and mostly in view of the indirect and genetic evidence, I prefer the hypothesis that the second cytochrome c is the controller or, if preferred, the repressor of the synthesis of the first cytochrome c (of the continuing one). As a matter of fact, it is not the cytochrome c as such but the new polypeptide chain of the iso-2 cytochrome c that is the controller of the synthesis of the functional type, the iso-1. The arguments are, as I said, indirect but there are three predictions from the evidence. One is that the model of the control is analogous to the classical model, except that it specifies that the repressor is nothing but a polypeptide chain strictly identical (by the usual criteria) to the functional polypeptide chain. We can predict the following: When we have a completely repressed cell having no

cytochrome c at all, the nude polypeptide chain of the iso-2 is sitting in the cell and it is repressing. On the induction we have, of course, the iso-1 synthesized, which means that iso-2 went away from the repressing site.

BAUTZ: How is the induction brought about?

SLONIMSKI: By oxygen. An easy way of doing this is to add heme and pull it away. But the prediction is that if we do an isotopic labeling experiment and if we first catch 50 molecules of both iso-1 and iso-2 synthesized, (which are both synthesized simultaneously) one is synthesized by adding heme and pulling it away, and the other is synthesized de novo from heme. In such an experiment, the molecules of iso-1 should be completely labeled and the molecules of iso-2 should be made from a high molecular product. This experiment has been done, and it has given this response. While we could not at first catch 50, 15 or 20 molecules per cell, we have done it at a level of about 200 molecules per cell and obtained the answer that iso-2 was 15 percent labeled, relatively speaking, while iso-1 was more than 90 percent labeled; thus we had a factor of 5 to 6.

The second prediction is that there should be mutations in which either not having iso-2 or having a diminished amount of iso-2 should increase the synthesis of iso-1 (and there are such mutations). If this is so, then there is a regulatory system of some kind. If we want to extrapolate and speculate on this kind of model, for every function of the polypeptide chain there is another almost identical polypeptide chain which will play a rather different role.

GREEN: Would you predict that the repressor, as you call it, would not be in the mitochondrion, then?

SLONIMSKI: I do not know where it takes place. I do not want to——

GREEN: It would not be much use in the mitochondrion, would it?

SLONIMSKI: What I can catch is only the iso-2 cytochrome c; that is, with the heme attachment. What I call the repressor is the nude polypeptide chain which preexists the induction. If we have a labeled precursor and if we use the proporphorin, we should be able to catch it. That is what we are trying to do, but it is difficult. Whether it is on the mitochondrion or not, I do not know. In an anaerobically grown cell, I do not have a mitochondrion in the sense that I see something—a bag with a double membrane and the crystae. I have a corresponding amount of proteins that I make in an ultracentrifugation separation. I also get something in the region that says whether it is mitochondrion sediment.

What I wanted to say, and this is purely speculative, is that perhaps during evolution what was important, as in the case of the minimum cell of Dr. Morowitz, was to get the cell growing more and more efficiently, and to get it growing more efficiently means regulation. If this is a type of regulation—that is, making polypeptide chains that are almost identical—this would mean setting more and more genes very similar in structure to the first function.

This is like Parkinson's Law: The way to create complexity is to have more and more employees in an office, with the result that the efficiency per employee is lower and lower; thus the only way of losing information, once we have it, is to get a new information to prevent the first information from working. But we cannot lose information, as such, in the sense that we cannot lose a piece of a genetic information which has been coded. Of course, there are deletions; no doubt there are deletions, but I do not think that deletions are selected by evolution, which would explain why we have such a tremendous amount of DNA. The amount of DNA per cell in a mammal is at least 1000, if not 10 000, times greater, not only in a PPLO cell but also in an E. coli cell. Nevertheless, the amount of protein is different; the enzymes certainly are not 10 000 times greater, which would mean that if all this speculation has any sense, probably 99 percent, or maybe even more, of the genes are the genes

which are regulatory in function. Thus, we are losing function by making more inhibitors of cell function.

DANIELLI: I think this is certainly a very provocative series of remarks, but I do not think we should attempt to discuss them tonight because the hour is too advanced. The time has come, therefore, when we should break off. I want to ask that, whatever may happen in the way of informal discussion in the course of the next few hours, discussion on this interesting theme should continue tomorrow.

2. Synthesis of Organelles
Discussion leaders: D.E. Green, S. Fox

DANIELLI: We began talking on the organelle aspect of this program yesterday. I think we ought to thank Dr. Slonimski for presenting an interesting set of data and speculations and also for doing so at what I guess was something like 3:00 a.m.

After discussing the way in which we should conduct today's session and talking to people who would like to participate, I thought it would be best if we immediately moved on to discuss the structure of organelles, particularly the membranous ones, and then go into the question of specificity and formation of organelles.

As I was pointing out, the structure we have to deal with in the case of certain organelles is two-dimensional. However, there are also, of course, many, many organelles that might conveniently be thought of as one-dimensional units—such organelles as spindles, muscle fibrils, and flagella, which might be regarded as assembled from linear arrays. By comparison other units such as mitochondria, chloroplasts and plasma membranes might be more correctly regarded as two-dimensional units. Some interest might arise from considering the different problems arising from those two types.

In the case of the membranous unit, the specificity of function is probably associated with permeases, receptors and such other macromolecules as are associated with the membrane. Yesterday I put in a plea, for example, for retaining the word "permease" to describe the macromolecules specifically associated with the transport phenomena in the membrane. It is a word without any very precise meaning and it is quite appropriate because we do not have any very precise information about these things. I think it is better to keep a rather indeterminate term to describe the macromolecules and their functions until they have actually been isolated and we know their functions exactly.

It could be that those people working on the mitochondria have come up with a system which can be more precisely defined, and it will be interesting to hear about that.

There are a number of problems that I have not space to write up on the board but which I will briefly read out. First, in these membranous units, are we correct in thinking that lipid performs a relatively nonspecific function and that the whole of the specificity resides in the macromolecules?

Second, I think we can say that it would be relatively easy to develop a physical theory for the structure of the lipid phase in these membranes, and I would like to suggest that that should be one of the endeavors in the field of theoretical biology.

Third, to what extent do the lipids and macromolecules interact to form specific lipoproteins? Supposing they do, the question arises which was put forward by Dr. Ycas: Do the lipids come first and then determine which macromolecules assemble into the membrane, or do the proteins come first and determine which lipids are assembled into the membrane?

Fourth, I want to point out that we do not know what the degree of interaction is across this type of membrane, but we do have to remember that the dielectric constant of the membrane is probably on the order of 3, at least in the lipid layer. As a result, if we consider

molecular attraction between the two macromolecules on opposite sides of the membrane, they are really much "closer" together than would be thought. This 40 angstroms of hydrocarbon is equivalent to about 6 angstroms of water. Therefore, we must consider seriously the forces acting across the membrane. This is a problem which has not been adequately studied from a theoretical point of view.

Fifth, I would like to raise the question of the forces acting between two such sets of membranes. This is in itself quite a complex problem. I do not suggest we settle down to discuss all these hypotheses at the moment. Instead, I am going to ask Dr. Green to present us with concrete data on mitochondria. I suggest that we bear these five points in mind as we enter into the discussion during the day.

GREEN: We thought it might be appropriate to begin a discussion of membrane systems by reviewing some recent developments in the study of the mitochondrion. Therefore, I am going to point out the kind of evidence that has been used to build up this picture and also some of the outstanding problems that would be of interest to a theoretical biologist. I think the mitochondria is frequently conceived of exclusively in terms of one function; namely, the capacity to convert oxidative energy into the bond energy of ATP. This transduction is, of course, its fundamental property but there are indeed other equally important properties (ref. 37). For example, it has the capacity for ion accumulation, and when I talk about ion accumulation I mean energized ion accumulation, and that brings the mitochondrion at once into line with all other membrane systems. It also has the property of contractility and carries on a series of synthetic reactions. Thus the mitochondrion is more than just a device for converting oxidative energy to the bond energy of ATP.

I would like to develop the thesis that the mitochondrion is basically a membrane system (ref. 38). While its primary function is indeed this coupling of electron flow to synthesis of ATP, it is a prototype of membrane systems generally, and many of the properties which I shall describe—structural properties as well as functional parameters—are applicable in large measure to other membrane systems.

GRENELL: Could I just ask Dr. Green if, in the light of that statement, he would care to suggest any specific differences between this general representation of membranes and an excitable membrane?

GREEN: No. In many cases we cannot define with great precision what is meant by an excitable membrane, but we can conceive of the membranes, for example of a nerve cell, as no different in character than a mitochondrion from the standpoint of structure. The difference may lie entirely in the primary function, but I think it might be easier to answer your point as we go along and consider the general properties of membrane systems—properties that are not unique to the mitochondrion.

FREMONT-SMITH: Does that mean we really will get the point answered, or does it mean it will be elided? Because this frequently happens, quite unconsciously, when somebody says, "Let me take it up later." The time when a question needs to be answered is at the time when it is asked.

GREEN: I believe it is implicit in the development; if it is not implicit, I hope you will do me the favor of asking the question again.

FRIEDENBERG: Will the permeability characteristics of this membrane system be treated as well?

GREEN: Yes, I will deal with those as we go along. But before we deal with the functional parameters, we will have to consider some morphology. The two things, of course, are inextricably connected, but I will try to deal with them separately for reasons of simplicity.

I think we can conceive of the mitochondrion as a structure made up of the following elements (ref. 17): (1) an outer layer that encompasses the entire organelle and delimits the organelle from the environment; (2) an inner layer separated from the outer layer by a space; and (3) a network or matrix system of protein and lipid, which is probably attached to the inner layer. Let us consider first the two-layered arrangement of the mitochondrion depicted diagrammatically in figure 13. The outer and inner layers are both chemically and structurally distinct. They fulfill different enzymatic functions. The space between the two layers contain a fluid in which we have reason to believe are present the various cofactors and ions essential for the activity of the enzyme systems associated with each of the two layers. The enzymatic events that take place in the mitochondrion are localized at different sites and the layer arrangement and geography of parts are relevant to the logistics of these mitochondrial events. The diagram shown in figure 13 does not resemble the picture of the mitochondrion all of you are familiar with. If we infold the inner layer in the form of tubular invaginations or cristae, the classical form of the mitochondrion becomes recognizable (fig. 14). Notice that the space within the cristae is continuous with the space between the outer and inner layers. The proteins of the inner layer and of the matrix layer are chemically linked to lipid predominantly in the form of phospholipid (ref. 39).

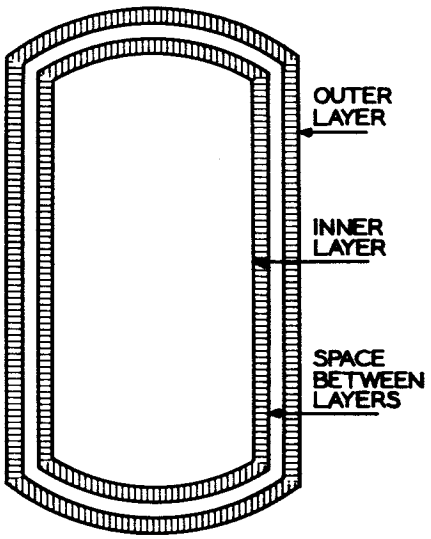

Figure 13.—Two-structured layer arrangement of the mitochondrion.

DANIELLI: No cholesterol?

GREEN: The amount of cholesterol is negligible in mitochondria, and it is really not relevant to our purposes at the moment. About 95 percent of the lipid is in the form of phospholipid, and the total amount of cholesterol is on the order of 1 or 2 percent and is really of no moment as far as the mitochondria is concerned.

Let us make a balance sheet of the amount of protein distributed between the two layers and the matrix (ref. 40). The matrix accounts for some 60 percent of the total protein whereas each of the two layers accounts for about 20 percent. The protein to lipid ratio is about 7:3 in the inner layer and in the matrix, but it is somewhat less in the outer layer (not all the proteins in this layer are linked to lipid).

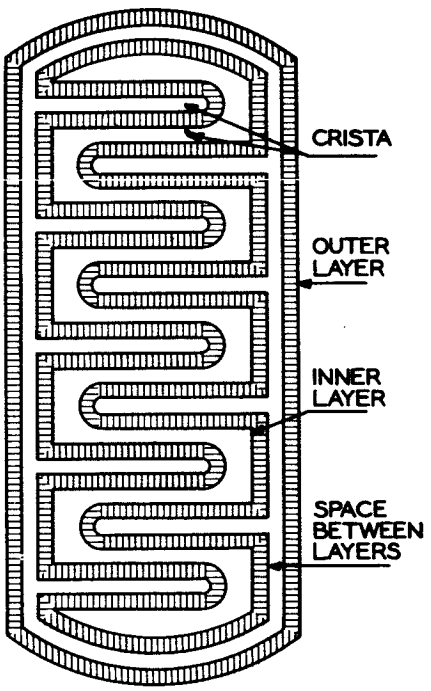

Figure 14.—Infolding of inner layer to form cristae.

The bulk of the mitochondrial protein is contained in the matrix layer—a layer which we have reason to believe is localized in the interior of the mitochondrion (i.e., on the interior side of the cristae and the inner layer). This matrix layer is a network of three polymeric proteins, structural protein, and the two contractile proteins—actin and myosin (ref. 41). The monomeric species of each of these three

polymers is of relatively small molecular weight. The monomer of structural protein has a molecular weight of about 22 000. The three polymeric proteins are cross-linked into a contractile network system.

From high-resolution electron micrographs (refs. 17 and 18), it has been deduced that both the outer and inner walls are made up of particles that are bonded together to make one continuous layer. The particles of the outer wall are made up of a set of enzymatic complexes, which appear to be involved in implementing the citric acid cycle, fatty acid oxidation, the oxidation of β-hydroxybutyrate, and some facets of lipid biosynthesis (synthesis of fatty acids and phospholipids). The particles of the inner layer are known as the elementary particles, and these contain the complete electron transfer chain as well as the apparatus for coupling electron flow to the synthesis of ATP. I am not going to say much about the particles on the outer wall because we have not as yet examined them in any systematic fashion. The particles on the inside wall have been very carefully studied, and we are beginning to understand a little about their fine structure.

Consider a single crista and the picture which emerges when this is examined by high-resolution electron microscopy after staining with phosphotungstate. My colleague H. Fernandez-Moran (ref. 17) of the University of Chicago and our group have collaborated in this joint venture over a period of 3 years. The individual particles that make up the wall of the cristae have a tripartite arrangement (fig. 15). The three parts are (1) a spherical head piece of 80—100 angstroms in diameter; (2) a cylindrical base piece 45x115 angstroms; and (3) a cylindrical stalk connecting the head piece and base piece (35x55 angstroms). Note that the fused base pieces of the elementary particles make up the wall of the cristae. I should mention that the elementary particles can exist in two forms—a tripartite arrangement as just described and a compressed form which is that of a sphere 150 angstroms in diameter.

STARR: What is the location of the adjacent headpiece on the adjacent crystal, just roughly—the next crystal over from the one in figure 15.

GREEN: There would be a space separating the elementary particles on one crista from the corresponding particles of adjacent cristae.

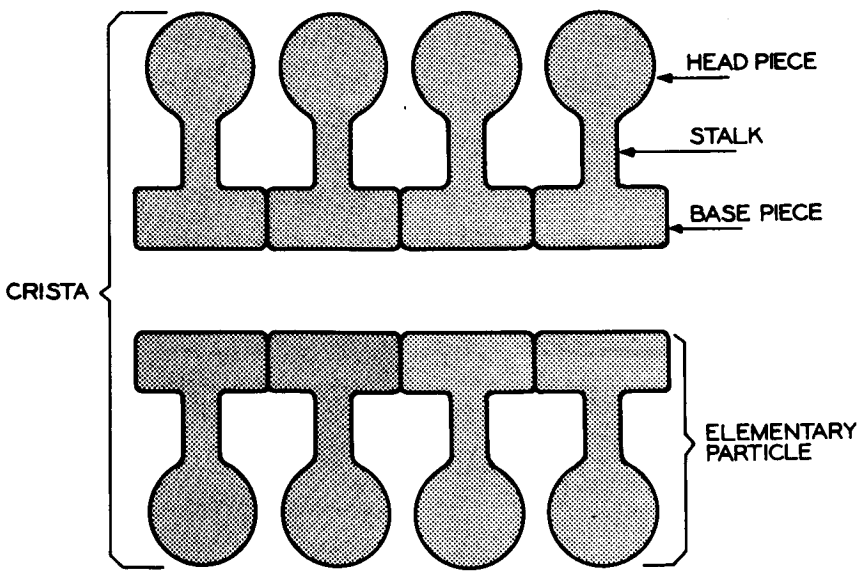

Figure 15. —Arrays of elementary particles in a crista and the three parts of the elementary particle.

STARR: A regular space—80 to 100 angstroms, roughly?

GREEN: I am glad you raised that question. When we look at the electron microscope photographs (ref. 17) of mitochondria that have been fixed with osmium, we have the impression of extraordinary regularity; they are arranged like soldiers in rows and all the cristae seem to have very precise dimensions. When phosphotungstate is used as a fixative, which shows up the structure very nicely, we do not see this regularity. The cristae are very irregular in width. It is a rather extraordinary business that, as we see more and more structure, more and more irregularity of the overall contours of the mitochondrion emerges. Therefore, to this question of how far apart are cristae or how wide is the interior of a crista, it is not possible to give a precise answer. We find great variations.

Now, what is especially noteworthy is that the outer layer of the crista is made up of the fused base pieces of these elementary particles. In other words, one layer of the membrane—if you like, the inner membrane—is, in fact, an essential part of this elementary particle; thus, we cannot distinguish between membrane and associated particles because the membrane is a fusion of the base pieces of the elementary particle.

Where is the structural protein? In this regard the electron microscope as yet has not been very helpful. We know that there is some type of network material in here, but how to describe and characterize it is still a mystery and more work has to be done in defining it. All we can say from available evidence is that it must be concentrated somewhere in the interior. What it looks like and how it is actually connected with elementary particles, we have no idea.

I would like to say a few words now about the electron transfer system of the mitochondrion. From the observed dimensions of these three parts, we can calculate what the molecular weight would be. That would be a simple calculation based on the known density of the various parts of the mitochondrion, which would be about 1.25 and the observed volume of the three parts. From the total volume and the density, we can calculate the molecular weight. The molecular weight, so calculated, has a value which lies between 1.3 and 1.4 million. That would be the dimensions of the electron transfer chain if we assume that the electron transfer chain is, in fact, associated with these particles. We can isolate from the mitochondrion a unit of about that molecular weight, actually about 1.4 million.* When we isolate that unit, it is not a tripartite unit but a sphere of 150 angstroms in diameter, of the correct size to match a unit 1.4×10^6 molecular weight.

I do not want to go into all the evidence, but we think that there is a good body of evidence to justify the identification of the electron transfer chain with these particles on the inner wall. Perhaps the most cogent bit of evidence is that if the mitochondrion is jiggled with sonic irradiation, the particles of the outer wall are dislodged as the external membrane is broken. But all the elementary particles associated with the inside wall are unaffected by sonic irradiation and remain attached to the cristae. These fragmented cristae still retain the complete electron transfer function and still retain these characteristic particles while all the other particles have been dispersed and left in the supernatant.

We know something about the arrangement of the electron transfer chain that will permit us to see a very important relationship between the structure of the chain and the structure of the elementary particle as visualized by the electron microscope. The electron transfer chain, in essence, can be conceived in the following way: It is a chain in which electrons move from DPNH to oxygen or from succinate to oxygen, and it consists of four complexes. We can represent it as depicted in figure 16. The electrons move through a set of complexes in the order shown. In each complex are bunched a set of oxidation-reduction proteins. We designate the various complexes by roman numerals (I-IV). The molecular weights of these

*D. E. Green, H. Tisdale, and A. Tzagoloff, unpublished studies.

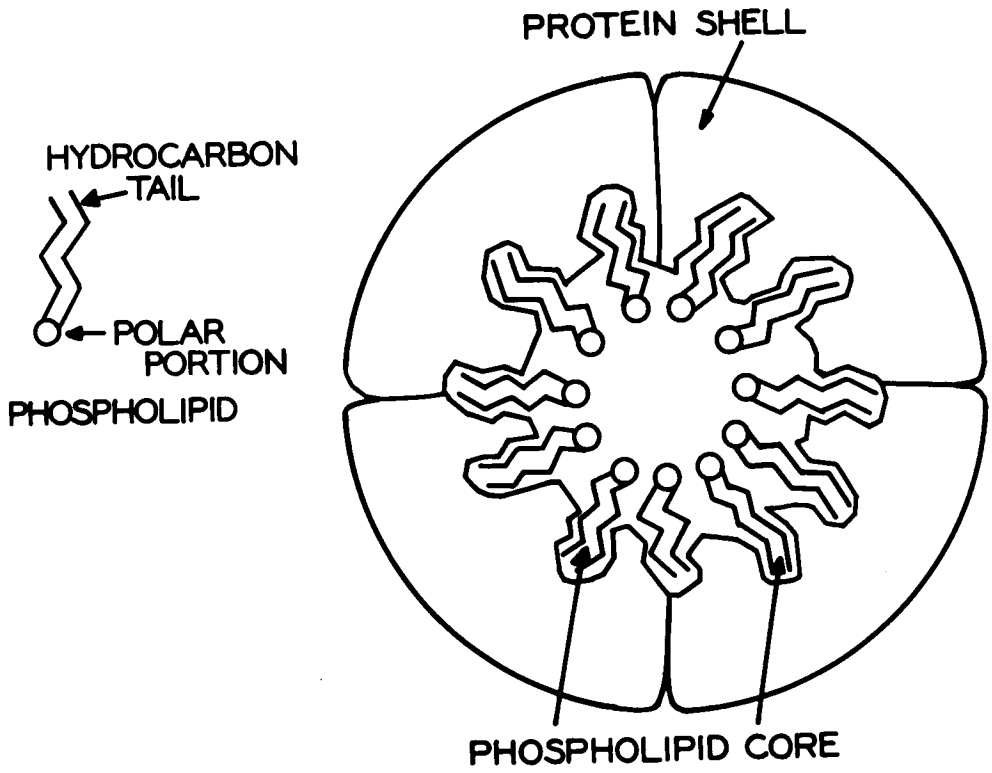

Figure 16.—Diagrammatic representation of a complex of the electron transfer chain. The protein shell (on the periphery) consists of four proteins hydrophobically linked one to the other. The inner edge of the protein shell is covered with phospholipid. The fatty acid residues of the phospholipid interdigitate with the hydrophobic side chains of the amino acids in the proteins. Not shown in this diagram are the functional groups of the protein that are oriented in the lipid core.

complexes are known and they will sum to a value of 1.4 million. Let us say that each complex accounts for roughly one-quarter of the total molecular weight of the electron transfer chain.

The structure of these complexes is of great interest to the group here.

POLLARD: Would you say that is three-quarters of a million total molecular weight?

GREEN: Yes, 1.4 million is the molecular weight. Neglecting the fact that the mass is not uniformly distributed among the four complexes, we may say that as an order of magnitude each complex has a molecular weight of from 3×10^5 to 4×10^5.

GRENELL: This includes everything—the oxidative phosphorylating things and cytochromes and the whole works?

GREEN: This includes everything that is pertinent to the primary transduction.

From studies which we have carried out (ref. 42) on the coupling proteins required for the terminal stages in the synthesis of ATP, we estimate that the total contribution of these proteins to the mass of the elementary particle would be less than 10 percent, and even smaller if these proteins are dissociable from the particle, as appears to be the case.

The electron microscopic study of the individual isolated complexes of the electron transfer chain has as yet not been carried out thoroughly (ref. 17). Our impression is that the complexes are spherical in shape and that the interior of these spheres has a different electron density from that of the periphery. We interpret this bimodal appearance of the complexes in terms of an outer shell of protein and an inner core of lipid. Our proximate calculations indicate that the amount of lipid present in each complex is insufficient to cover the periphery of the sphere which corresponds to the complex and that an interior position for the lipid would be mandatory. The various proteins present in each complex would be bonded one to the other in the outer shell (fig. 16). This whole problem of lipid-protein packing in macromolecules should be of intense interest to the theoretical biologist.

QUIMBY: Are you talking about the primary particle in the inner layer?

GREEN: I have just been referring to the electron microscopy of the individual complexes of the electron transfer chain. I have yet to relate these complexes to the three parts of the elementary particle. We are considering now the electron transfer chain that we can isolate from the mitochondrion by classical chemical methods. We have a unit of molecular weight, 1.4 million. We can resolve it into these four segments (refs. 37, 43 and 44) roughly equal in molecular weight, and we are now concerned with the structure of the individual complexes of that chain.

POLLARD: Is that one of those segments?

GREEN: That is right. Figure 16 shows one such complex. It would be I, II, III or IV. What I am saying now has general applicability to all four complexes, although our most definitive evidence comes with complex IV where we can, in fact, see in the electron microscope the doughnut character of the complex.

GRENELL: Has the molecular weight problem been settled, actually? There was, as I remember, some difficulty about this.

GREEN: Yes, there has been a serious difficulty in regard to the molecular weight of the elementary particle, but I believe this has now been satisfactorily resolved. The estimated molecular weight of the particle seen in the electron micrographs and the determined molecular weight of the isolated particle containing the complete electron transfer chain both come to the same value—1.4×10^6.

YCAS: Is this precisely isolated? How is it broken up?

GREEN: That would be a long story. The bonds that hold together the four complexes of the unit containing the electron transfer chain are predominantly lipid-protein bonds, which are hydrophobic in character. Bile salts in combination with salts such as ammonium sulfate or acetate have proved to be the most effective reagents for rupturing these bonds.

MOROWITZ: The structure represented by the line in the right-hand drawing (fig. 16)—what is that?

GREEN: The fused base pieces of the elementary particles constitute the wall of the crista. The crista can be conceived of as a hollow tube, the walls of which are made up of the base pieces of the elementary particles. In my line drawing of the crista, I represented the wall by a line.

MOROWITZ: Is this true of the outer membrane also?

GREEN: The particles on the outside wall or layer of the mitochondrion have not been thoroughly studied by us. We can say with confidence that the particles of the outer wall do not show a tripartite arrangement as do those of the inner wall.

FOX: Is the description here an extension of your earlier statement that the components of the double layer differ chemically? From the simple depiction in the first instance of a double layer you have made some changes, and I am wondering if they are an elaboration of that first statement.

GREEN: If, as we have reason to believe, the particles of the two walls are not identical, then there would be corresponding chemical differences. For example, none of the functional groups of the electron transfer chain would be present in the outer layer. In addition, the property of ion translocation applies to only one of the two layers.

FOX: That is a functional difference?

GREEN: Admittedly the capacity for ion translocation is a functional attribute of a layer and not a chemical property, but this capacity has to be referred back to chemical structures.

FOX: What do you know, specifically, about the difference, chemically?

GREEN: The main difference is that the particles of the outer wall fulfill a different enzymatic function than do the particles of the inner wall. That is to say the enzymes on the two walls are different.

FOX: How do you know it is different chemically?

GREEN: Shall we put it this way? If the two walls have different enzymatic properties, if there is a property in one wall that is not present in the other, we are so bold as to assume that this is related to the chemical composition. Would you agree?

FOX: Surely, I would agree, but I interpreted from your initial statement that you had some chemical specification that could describe this difference.

GREEN: We cannot isolate pure outer wall or pure inner wall and do chemical analyses. We infer the chemistry of the walls from the nature of the associated enzymes. The difference in enzymatic composition is our basis for postulating a chemical difference.

FOX: That answers the question. Thank you.

YCAS: Dr. Green, by that are you implying that the lipid is in the structure on the left of figure 16? Does that account for the total lipid in the inner layer?

GREEN: Lipid is uniformly distributed throughout the mitochondrion except for some of the particles associated with the outer wall. Apart from these exceptions noted, the ratio of protein to lipid is about 7:3 throughout the mitochondrion (ref. 45).

MANILOFF: Are these hexagonally arranged? If we looked down on this instead of at a cross section, would we see hexagonal close packing?

GREEN: That is right. We would see a mosaic of these headpieces and we do, in fact, see such.

MANILOFF: There are no holes, then, in this?

GREEN: Holes?

MANILOFF: Areas of the surface that are not covered by these base spherical things.

GREEN: When the crista is seen in top view as a mosaic, there is of course a space between the adjacent headpieces that make up the mosaic.

MANILOFF: How about the base ones? Do they fill completely the entire cell?

GREEN: I believe that the available electron micrograph evidence gives no encouragement to the notion that there are holes in the walls of the crista or of the outer wall.

GARFINKEL: Do the elegant membrane particles contain the hexokinases that are sometimes found associated with mitochondria?

GREEN: We really have not studied that particular point.

MOROWITZ: What is the correspondence between the picture you show and the usual picture of the sandwich membrane?

GREEN: Let me put it this way. The sandwich arrangement of the crista applies regardless of which fixative is used. By that I mean we see two densely staining outer layers separated by an electron-transparent region between these two layers. What is variable when different fixatives are used is not this three-layered arrangement but the dimensions of the structures or particles making up the outer layers of the sandwich. In addition, there is variability in the width of these layers as seen in electron micrographs of specimens fixed in different ways. We explain the change in substructure of the layers in terms of the depolymerizing action of certain fixatives such as osmium tetroxide. Stoeckenius has recently demonstrated that the elementary particle virtually disintegrates into smaller units when exposed to osmium tetroxide, and this transformation is probably a consequence of rupturing the bonds between the subunits that make up the particle. I consider osmium as a scrambling reagent.

McMULLEN: Does not phosphotungstate also scramble somewhat in the sense that phosphotungstate is not such an active fixative? It is more of a negative staining technique. Why should the structure determined by phosphotungstate be any more definitive than that determined by osmium tetroxide?

GREEN: The reasons are purely chemical. Phosphotungstate is a relatively mild reagent. It reacts electrostatically with particles such as the elementary particle, and this interaction is reversible. When phosphotungstate is removed from the particle by repeating washing, full enzymatic activity is restored to the particle. I consider this a fairly good indication that no violent changes have taken place in the particle. There are no precedents in enzymology for retention of activity in catalytic proteins that have sustained profound structural changes. Thus, I believe it is justified to consider retention of activity as prima facie evidence that no major structural changes have taken place in the proteins. By this criterion we can say that osmium must be inducing profound structural modification since it completely and irreversibly destroys enzymatic activity.

MANILOFF: Are you then saying fixatives that retain enzymatic functions are, by definition, good fixatives for morphology?

GREEN: As a rough guide, yes; the less damage done, the more reliable the results are likely to be. For example, as I understand it, the viruses are still viable after interaction with phosphotungstate.

DANIELLI: I think there is a point here, Dr. Green. To fix, we are obliged in some sense to solidify the structure.

GREEN: Not quite. Not with this reagent. That is just the beauty of it. The phosphotungstate coats the particle, and what we see is the structure within the densely staining coat.

DANIELLI: How do we know that the structure inside is the way it was originally? You say that interaction between the phosphotungstate and the additional structure is primarily electrostatic. It seems to me that in a system like this there is a considerable risk of setting up phase changes with redistribution of the particles which remain, shall we say, intact as far as their internal structure is concerned, but which are rescrambled into different aggregations. If I understand the present evidence correctly, when we unscramble the whole structure

and get separate units (I, II, III, IV), we could not possibly define what the original relationship was between these and the membrane. All we know is that it makes sense biochemically to have these units aggregate into one body, but we do not know whether the spatial relationship between the separated fractions is the same as the spatial relationships in the original membrane.

GREEN: Yes. Well, I would say that we are relying on the evidence of the retention of the enzymatic activity. This is our most powerful bit of evidence and, to my way of thinking, if you want to contend that this is not a good indication of the state of the system, you will have to come up with a better criterion than enzymatic activity. You are opposing a very powerful bit of evidence by a vague intuition that something might happen, but this intuition is not based on chemical evidence. You suspect that things might happen. We would take the position that since enzymatic activity is fully retained in all its essential characteristics we can infer that no basic morphological changes have taken place.

FORRO: There is at least one piece of evidence that can be thought of in terms of enzymes that will actually work in crystalline form. I know that ribonuclease can be kept crystallized in ammonium sulfate and work on small substrates. Here is a morphological change into solution of individual particles in one case, and in the other they are related to each other in crystalline variety.

GREEN: Well, yes, but crystallization would not be equated with unscrambling of the structure, would it?

FORRO: No, but there can be a change in form and retention of function.

GREEN: That sort of change, I think, would be——

POLLARD: How is the form changed on crystae?

FORRO: That is the $64 000 question—whether the internal structure of the individual molecule is altered at all.

POLLARD: Is any change expected?

FORRO: I do not know. In fact, I think that is what these experiments are being conducted for.

POLLARD: What would have to be based in crystals? I cannot quite see why the aggregation of a set of molecules together means that the molecules are altered.

FORRO: No, I have misstated the point. I had a slip in reasoning. The experiment is being done for another reason, and that is to see whether or not, in fact, in the crystalline form there is a difference in the internal structure of the molecules, whether or not they are acting on substrate. I would not have any good guess as to whether the three-dimensional form is different in solution than in the crystal. The point I was making was that we can have a different physical state, essentially, and still have activity. Therefore, it seems to me that the reasoning that, because we have activity preserved, this automatically guarantees that the physical state is the one that was there originally is not valid.

GREEN: Perhaps I can put it more precisely. I think there is a range of fluctuation in protein form that is compatible with enzymatic activity. Within that range, we cannot say that no change has taken place; but the rescrambling, the complete change in protein state, is, I think, interdicted when there is full retention of enzymatic activity. I would like to hear of a protein which undergoes profound structural change without loss of enzymatic function.

DANIELLI: I do not think that is really the issue we are questioning you about. The issue was whether the relationships between the individual macromolecules were changed as the result of introducing phosphotungstate or other agents.

GREEN: Are you proposing that we could, in fact, modify the relationship of the protein to this lipid without modifying the enzymatic activity?

DANIELLI: I am sure we could.

GREEN: I am saying that if the evidence is produced I shall bow before it.

DANIELLI: I will not worry about that now, but it can be done. I have not the slightest doubt about it.

GREEN: The fact that you do not have the slightest doubt is really not the relevant issue. The issue is, what experimental evidence is there that this would happen?

DANIELLI: I do not think this is quite the best way to discuss it. The point is that I am quite sure we can find a system in which we can adsorb a protein on a lipid surface and have its specific activity retained. In fact, in the case of lipose, we know——

GREEN: Ah, I see what you have in mind. You are thinking of this lipid as something that has gone along for the ride, that the catalytic element is the protein.

DANIELLI: No, no!

GREEN: We think of particles such as the elementary particle as lipid-protein systems—systems as precise chemically as protein systems. In fact, if lipid is removed from the system, there is complete loss of function. Although the protein is unmodified by lipid extraction, the mere removal of lipid leads to loss of activity. When lipid is reinserted into the system, activity is fully restored. The intramolecular relation of protein to lipid is a very important and precise one.

FREMONT-SMITH: Important for the enzymatic function?

GREEN: Yes, all the enzymatic function is lost when lipid is removed. This is not a chance relationship of protein to lipid.

YCAS: How do you remove the lipid, Dr. Green?

GREEN: By extraction with acetone and water, 90 percent acetone and 10 percent water (ref. 46). At low temperatures we can remove the lipid completely and activity is lost. Then we can reinsert the extracted lipid and activity is restored.

POLLARD: I think we are doing exactly the wrong thing. We had Dr. Green going very beautifully, and he was going very nicely toward directions that would help us in theoretical biology. Now we are asking about all our little anxieties. Let us leave our anxieties alone, because this is a picture which we will not get again. If we interrupt Dr. Green, we are not going to get the finish of the story but are going to wind up with our anxieties. I would like Dr. Green to continue.

GREEN: Thank you. Not that I am unwilling to answer some of these questions but, as you say, the continuity is often lost and there are some quite exciting——

DANIELLI: Let us just get this tied up.

SZENT-GYORGYI: Will you correlate the three pictures with a word?

GREEN: The correlation will take more than one word. We have to relate two particles—one that we see in the electron micrographs (the elementary particle) and one that we isolate and in which the electron transfer chain is localized. The elementary particle has a tripartite arrangement; the isolated particle consists of four complexes. The key questions are: (1) What is the identify of these two particles? (2) If identical, can the three parts of the elementary

particle be identified with the complexes? I have already discussed our principal lines of evidence for equating the two particles—identify of molecular weight and invariant association in submitochondrial particles. If we can assume that the observed and isolated particles are identical, then the four complexes of the chain will have to be distributed among the three parts of the elementary particle. Provisionally we would assign complexes I and II to the base piece, complex III to the stalk, and complex IV to the headpiece. The fits are reasonably good except for the fit of complex III and the stalk. The dimensions of the stalk are not large enough to accommodate complex III. However, there is an uncertainty about the third dimension of the stalk (only two dimensions can be measured in the electron micrographs), and this could be sufficiently large to resolve the discrepancy.

The packing of lipid and protein in the elementary particle and in the matrix layer poses one of the most fascinating problems of the mitochondrion. In fact, the mode of packing of protein and lipid and the consequence of this molecular marriage in terms of function are aspects which go to the very heart of the structure-function interrelationships of the mitochondrion. The hydrophobic interpenetration of a protein by phospholipid poses some intriguing three-dimensional problems, which should be of the greatest interest to theoretical biologists.

Let me give an example of the kind of lipid-protein packing that we have to deal with in the mitochondrion. The matrix layer as well as the elementary particles contain 30 percent by weight of lipid in the form of phospholipid (refs. 39 and 40). When lipid is extracted from these systems by acetone under the conditions I specified previously, the electron micrographs (ref. 46) of such lipid-extracted particles do not register this massive change in the particle. The electron micrographs of the normal and extracted mitochondria appear indistinguishable. The form and size of the normal and lipid-depleted mitochondria are indistinguishable. Whether lipid is present or not, the mitochondrion looks the same in electron micrographs.

The take-home lesson to be learned from this demonstration is that the structural pattern of the mitochondrion is set by the protein and, equally important, the lipid is attached to the protein in such a way that removal of lipid does not disturb the relation of one protein molecule to another. We could readily account for this phenomenon if we assume that lipid is buried in the interiors of lipid-protein complexes whereas protein is localized in the outer shell. In the electron micrographs we see only, or predominantly, the shell of the macromolecular structures; thus the presence or absence of lipid may not be recognizable by inspection.

The mitochondrion is an extraordinary device for concentrating particular ions (refs. 44 and 45). Magnesium or calcium or manganese ions in concert with phosphate ions can be moved from the external medium into the interior of the mitochondrion essentially quantitatively. Under suitable experimental conditions the whole interior of the mitochondrion is laden with deposits of tricalcium or trimagnesium phosphate. I think it is an academic question to ask whether this is an active process in view of the completeness and magnitude of the transfer process.

How are these ions moved? I think we can specify with considerable confidence some features of the mechanism. This is the first system involving ion transport that has lent itself to resolution in biochemical terms. Each pair of electrons that moves through the electron transfer chain generates three high-energy intermediates. Each such intermediate on the way to ATP can support the movement of two atoms of the divalent ion and one molecule of phosphate. This is the basic stoichiometry of the translocation. The translocated ions are deposited in the interior of the mitochondrion (probably in the matrix layer) in the form of $Mg_3(PO_4)_2$, $Ca_3(PO_4)_2$ or $Mn_3(PO_4)_2$. For each molecule of phosphate deposited, one hydrogen ion is released. Thus, acid formation parallels ion translocation. The energizing element in the translocation is the high-energy intermediate, and this is expended in the process of the translocation.

PITTENDRIGH: Could you give us those experimental facts again? For every high energy bond formed, two magnesiums and one phosphate——

GREEN: ——move in and are precipitated inside as magnesium triphosphate or calcium triphosphate. There is no specificity with respect to bivalent ions.

KLEIN: Is it that they can move in or that they always do? I am not quite clear on this. Is it under special conditions that this happens?

GREEN: Under ordinary conditions translocation is interdicted. As long as ATP can be synthesized, ion translocation is excluded. But if synthesis of ATP is repressed by reagents such as oligomycin, then ion translocation can proceed. Under suitable experimental conditions, it will proceed as rapidly as synthesis of ATP.

POLLARD: What agent is used?

GREEN: The most effective reagent we have found for suppressing ATP synthesis and permitting ion translocation is oligomycin—an antibiotic whose chemical composition is as yet unknown. This reagent does not affect the synthesis of the high-energy intermediate nor the interaction with the translocating system, but it prevents the interaction of the intermediate with ADP.

The studies we have carried out on the translocation process lead us to the following picture. In one of the two membranes or walls of the mitochondrion, there is a macromolecule that is the instrumentality of ion translocation (refs. 47-49). This macromolecule is vectorially arranged in the wall so that one end faces the medium containing the ions to be translocated and the other faces the interior into which the ions will be translocated and deposited. The molecular unit of translocase action combines with two atoms of the divalent ion and one molecule of phosphate. This is the loading reaction. The loaded translocase is energized by one molecule of the high-energy intermediate and undergoes a change such that the ions which were loaded on the exterior end of the translocase become transferred to the interior end where these are unloaded. Let us imagine that the interaction of the translocase with the high-energy intermediate leads to a conformational change of such a nature that the position of the loaded ions is shifted from an exterior to an interior position. I must repeat that we are dealing with strict stoichiometry in these events. The number of ions translocated is strictly proportional to the number of molecules of high-energy intermediate provided to the system.

Now we come to some of the geographical details. Where is the translocase localized? Our initial impression was that there was no choice but to localize the translocase on the outer wall of the mitochondrion. Evidence is multiplying, however, that the translocase is localized on the inner wall—the exterior edge facing the space between the two walls and the interior edge facing the contractile matrix layer. * The translocase may well be the elementary particle-matrix couple. The interaction of the high-energy intermediate with the contractile matrix system leads to a conformational change that is transmitted to the elementary particle by virtue of the chemical links between the elementary particle and the contractile system. The loading process proceeds in the elementary particle; the contractile process in the matrix. The contraction of the matrix may induce an expulsion of an involuted segment in the elementary particle. The expanded tripartite arrangement of the elementary particle could represent the state after contraction of the matrix; the compressed spherical form of the elementary particle could represent the state before contraction. The matrix network of the mitochondrion contains the contractile proteins actin and myosin, or at least the analogs of these proteins from muscle. We have grounds for believing that in all membrane systems

*P. Blair and J. Purdue, unpublished studies.

the instrumentality for moving ions and molecules in an active process follows closely the mitochondrial model.

GRENELL: Do you mean, Dr. Green, in all membranes examined under conditions of their normal functional state, or under your special chemical conditions which may not bear any relation to the real thing at all?

GREEN: From your question I take it you are dubious whether the events that apply under the conditions which we have found (ref. 38) to permit ion translocation in mitochondria are relevant to the counterpart events under physiological conditions. Furthermore, you are concerned whether it is permissible to extrapolate from mitochondria to other membrane systems. The only unphysiological component we use in our studies of translocation is oligomycin which, I might add, can be replaced by the parathyroid hormone in several mitochondrial systems. As to the generality of this mechanism of active transport in membrane systems, I would like to submit the following relevant set of facts. Wherever defined membrane systems have been studied in some depth, they have been found to share the following properties: a double-walled membrane arrangement with a space between the walls; a particulate character of the walls in the sense that the walls are made up of fused particles; the presence of a matrix system (structural protein plus contractile proteins); the capacity for ion translocation which runs parallel with an ion-sensitive ATPase activity; and the presence of lipid in the form of phospholipid accounting for at least 30 percent of the total dry weight. This seems to be a generalized structural and functional pattern for all membrane systems. I interpret this uniformity of pattern to mean the identity of the basic mechanisms, and this thinking underlies my readiness to extrapolate from the mitochondrial membrane to membranes generally.

GRENELL: Under these conditions, for example, are the same time relationships obtained for these fluxes as would be in other systems?

GREEN: The speed of these reactions?

POLLARD: He said he did. He said the same rate as the rate of synthesis of ATP, normally.

GREEN: In mitochondria, yes, but in other membrane systems, I could not say.

POLLARD: Do you have any idea how many of these contractile proteins would have to be on the surface to obtain the full flux rate? I mean quantitative relationship.

GREEN: We know the flux rate of ions per mitochondrion, but we do not know the number of translocases. We are up against the problem of defining the translocase unit.

POLLARD: No, no, only the number of ions. There is the stoichiometry of one phosphorus and two magnesiums. Now all we have to do is to count up the number of those packets that go in, and then we can start to argue about the number of translocases. We could assume that it is the normal rate that a muscle contracts, that the muscle fibril contracts, and we can use that as a basis for giving us the number per second. In this way, we could get some idea.

GREEN: I think a calculation can be made if we make the assumption that each elementary particle is a translocase. Thus, in a heart mitochondrion there would be about 50 000 translocating units, and each such unit would go through about 9 cycles per second, with each cycle involving the movement of two divalent cations and one molecule of phosphate. Thus, in one mitochondrion 9×50 000×2 or 900 000 atoms of divalent ion would be translocated per second at 38 degrees. As a rough approximation we might consider the unit of contractility as of the same mass as the elementary particle. Each contractile unit would thus undergo 9 cycles per second.

As I mentioned before, we are thinking in terms of the combination of the elementary particle and the contractile matrix as the translocating unit. The elementary particle

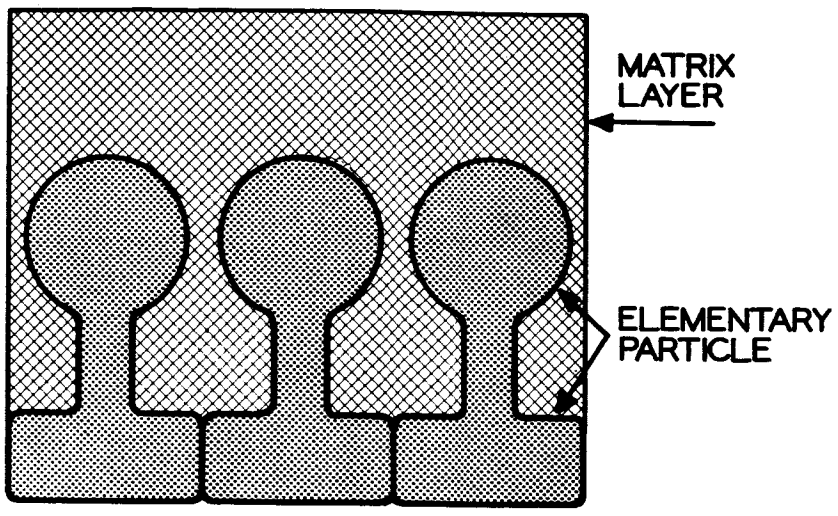

Figure 17. —Relation of the matrix layer to the elementary particles. A set of three elementary particles is shown with the matrix layer that envelopes these particles.

generates the high-energy intermediate which contains divalent ions (two per molecule of intermediate) and phosphate in high-energy link to the protein. The high-energy intermediate is a protein that is accessible to the contractile matrix enveloping the elementary particle on the interior end (fig. 17). The contraction of the matrix layer catapults the high-energy intermediate from the elementary particle into the matrix layer with disengagement of the ions (divalent metal ion and phosphate).

At this point I think a few words are in order about the matrix layer. As I mentioned before, this contains three proteins that form polymers—structural protein, actin and myosin. Ohnishi and Ohnishi (refs. 50-52) of Osaka University were the first to recognize the presence of an actomyosin contractile system in mitochondria and other membrane systems. This pioneer discovery has been verified in other laboratories and most extensively by Lester Packer and his group (refs. 53-55) at the University of California in Berkeley. The contractile system of mitochondria closely resembles that of muscle, but it is too early to say how similar or different the respective systems are.

What reasons do we have for invoking the contractile matrix layer in ion translocation? A vectorial ion movement having a stoichiometric character can only be conceived of in terms of a vectorial conformational change in a protein. The presence of a contractile system immediately surrounding the elementary particle can hardly be considered a happenstance. If there is validity to our interpretation, we should expect that the high-energy intermediate (but not ATP) can induce a contraction of the mitochondrial matrix system and that divalent ions should play some role in inducing this contraction. There is increasing evidence that divalent ions, particularly calcium, are essential for the ATP-catalyzed contraction of muscle actomyosin.

At any rate, a highly intriguing picture is emerging of ion translocation as a collaborative effort between a particle concerned with the generation of high-energy intermediates by coupled electron flow and a contractile system enveloping this particle capable of being energized by these high-energy intermediates. The elementary particle would be, so to speak, the gun fired by the contractile system—the shell being the high-energy intermediate. But this shell would also be the instrument for detonating the contractile system.

From considerations of the kind I have been discussing this morning—the system of layers in the mitochondrion, the relation of particles to these layers or walls, the interrelationship of lipid and protein, and the mechanism of ion translocation—we have the impression that we are beginning to see a unified pattern for all membrane systems. Obviously we are dealing with systems of a high degree of order as well as of complexity. The classical picture of membranes as structureless sets of layers is certainly invalid. Each layer of the mitochondrion shows a great deal of ultrastructure. Between the two layers of particles, there is a space with dissolved coenzymes. Beyond the inner wall or layer, there is the matrix layer that underlies the structural stability of the mitochondrion. This complex arrangement of parts within parts would not lend itself to spontaneous assembly. I am more and more inclined toward the view that the synthesis of membranes requires a precise ordering system, the nature of which I am not prepared to go into at the moment. It is sufficient to say that the starting materials for this postulated membrane system would be proteins and phospholipid micelles. I have been deliberately dogmatic about the possibility of spontaneous assembly of the mitochondria to compel attention to the new features of membrane structure that, in my opinion, lie beyond the capabilities of spontaneous assembly.

KLEIN: May I ask a question about those ultraparticles? Was it Parsons who reported some cylindrical-looking objects tangential to the surface of the mitochondrion?

GREEN: That is right.

KLEIN: How does that correlate with what you have seen?

GREEN: He probably knows more about those outer particles than we do. We have not studied them in detail but, undoubtedly, those are different from the elementary particles in the inner layer.

KLEIN: You also mentioned some biosynthetic activity. Could you give us one sentence on that?

GREEN: Yes, lipids. Some of the steps in the biochemistry of phospholipids take place in these particles on the outside.

PITTENDRIGH: Did you say something about the Kreb's cycle in the outside particles?

GREEN: They all appear to be associated with the particles on the outer layer.

PITTENDRIGH: What is known about the functional situation——

DANIELLI: May I interrupt? When you talk about the outside, do you refer to this surface?

GREEN: That is right, plus its membrane. I am afraid I have not defined terms and that is always a bad business. The outermost structure of the mitochondrion I shall define as a wall made up of fused particles no thicker than these particles. Thus, the designation "wall" is used synonymously with "layer" or "membrane." There may be components in this wall other than the component particles, but until these show up I shall equate the outer wall with a layer of fused particles. The inner wall is similarly defined as a layer made up of fused elementary particles or, at least, the base pieces thereof. It is the inner wall that by invagination gives rise to the cristae. To the interior side of the cristae or inner wall is the matrix layer. Between the outer and inner wall of particles is a space which presumably is filled with fluid. As long as the locale of the matrix layer was in doubt, this precision of definition was difficult. But now that the localization of the matrix outside the cristae seems quite certain, it is possible to define the different parts with a minimum of ambiguity.

DANIELLI: Are the Kreb's cycle enzymes associated with that particle or with some——

GREEN: No, no! They are associated with the particles that are attached to the outer wall or layer.

PITTENDRIGH: And that outer elementary particle of the outer mitochondrial membrane, to your knowledge, does not have the dumbbell structure you described for the particles of the interior?

GREEN: We have not studied in detail the particles associated with the outer layer. But there is general agreement in the field that the tripartite character of the dumbbell shape of the particles on the inner wall does not apply to the particles of the outer wall.

Part of the difficulty we have experienced is related to the idiosyncrasies of the technique used in staining with phosphotungstate. The specimen is squeezed on a grid and all the membranes are flattened. Under those conditions the distinction between outer and inner walls or membranes is blurred, and it is not easy to decide which layer is the outer wall and which the inner wall.

PITTENDRIGH: I can imagine that, but then I should think the same difficulty would apply to recognizing the inside membrane of the structure.

GREEN: The cristae are unmistakable; they are so clear because of their tubular arrangement. We see the edge only, of course, and not the interior.

PITTENDRIGH: Do you have any views on the functional significance of the fluid matrix between cristae?

GREEN: The space between cristae is filled with a matrix material which may be visualized as an aqueous suspension of polymeric material. What the solution in which the polymeric species are suspended consists of we have no idea at present. It could be an ultrafiltrate of the solution present between the outer and inner walls.

MOROWITZ: You said that you are very dubious about the possibility of this thing coming together in any kind of spontaneous way because of the great complexity. However, on the other hand, did you not indicate a partial experimental confirmation of this in the fact that if, under the right conditions, the lipid is taken out of the particles and things are just mixed back together the lipids go back and give a functional particle?

GREEN: That is right. Do you think I should be consistent and extrapolate further?

MOROWITZ: I do not know that you should extrapolate further, but I do think your own experimental evidence is perhaps indicating the way they do assemble without a great deal of——

GREEN: Yes. That is so fundamental a point that I would like your indulgence in explaining why I think this does not approximate the problem as I see it.

We can routinely reassemble the electron transfer chain by recombination of the four complexes (ref. 43) just as the virus can be reestablished by interaction of the coat protein polymer with the nucleic acid from which it has been separated. In this type of reconstruction, we are merely reestablishing bonds between two complex partners. This is a relatively simple fitting together of parts. We have broken bonds, leading to separation, and reestablished the original bonds, leading to reconstruction.

But this is a far cry from the complexity one would have to face in reconstructing, let us say, a complex from its component proteins and phospholipid. Consider, for example, complex III which is made up of two molecules of cytochrome b, one of cytochrome c, and one of nonheme iron protein, with these four proteins linked one to the other and to phospholipid. These four molecules are linked to one another and to phospholipid in a very precise way within the complex. But consider how the free monomer of the three different protein species would behave. Each one of these monomers alone would at once form an utterly insoluble polymeric network; together these would form mixed insoluble polymers. How would we imagine a spontaneous interaction of three hydrophobic materials that form polymers at the drop of a hat with one another or with any other molecule that is hydrophobic? There are

endless possibilities for hydrophobic interactions. If only one possibility is realized in nature, that would argue for directive influences operative in the assembly process. I find the fact of endless possibilities incompatible with the notion of spontaneous assembly. If some way can be dreamed up in which it would be possible to reduce this statistical interplay of molecules, I will accept it; but we have not found any escape from the dilemma other than to invoke an ordering mechanism.

McMULLEN: I think it is conceivable to think of ways, based partly on interfacial energy considerations and——

GREEN: Considerations of interfacial energy leave me cold.

McMULLEN: They do not leave me cold.

SLONIMSKI: I think Dr. Green has given a most beautiful description of the beef heart. What strikes me is the fact that what he calls the elementary particle with very striking and precise stoichiometry is something that, to me, is extremely variable in the sense that we can get all sorts—well, I can give 400 different types because I have 400 different types in my collection but, presumably, there are more of them. Nevertheless, it presumably has some kind of regular pattern in spite of the variability. So I do not know whether the term "unit" or "particle" is something that is proper; although its particle can be changed almost indefinitely, nevertheless it preserves its major characteristics.

GREEN: Let me specify the kind of evidence that will have to be produced before we can accept Dr. Slonimski's presumption of variability. Each complete electron transfer chain or elementary particle is made up of a set of four complexes. Each complex in turn is made up of a set of proteins—very precise in respect to the number of molecular species and to the total number of molecules of each species. At least that is the situation in a variety of electron transfer systems that have been studied in depth.

The overall stoichiometry of components in the chain is an expression of the fact that each of the four complexes has precise stoichiometry. Thus, in the chain of beef heart mitochondria (ref. 56) there would be for each molecule of succinic flavoprotein, six molecules of cytochrome a, 3 of cytochrome b, 1 of cytochrome c, et cetera. My point is simply this. It would be impossible to increase the number of molecules per chain of, for example, cytochrome a without completely altering the chain. Suppose that the mutant had 12 molecules of cytochrome a instead of 6. That would mean a new form of complex IV would have to be evolved; a new form of complex IV would mean an elementary particle of a different size; an elementary particle of a different size would completely alter the structural pattern of the mitochondrion. Changes in stoichiometry have to be compensated for by adjustments all along the line, each of which would require major structural modification.

The major features of the electron transfer chain are seen in all forms of life with remarkable fidelity and constancy. If in two billion years the electron transfer chain has resisted the erosion of the evolutionary process, it is difficult to believe that one mutation in a yeast cell could accomplish what two billion years of evolutionary development failed to accomplish. The issue now boils down to this. Are the changes in stoichiometry those established by analysis of the isolated particles or of the whole cell? Are these changes of a kind that would not necessitate a new architectural plan for the elementary particle? If it can be stated with confidence that the changes in stoichiometry apply to the isolated particles containing the electron transfer chain, I will have to bow before such evidence.

SLONIMSKI: We have it, because particles of around two million have been obtained from the different strains which are either regulated on the phenotypic level, that is by induction, or regulated by genetic mechanisms; and within the particles the ratios are different.

GREEN: Can you please specify the particular ratio of components you are measuring in these particles?

SLONIMSKI: Cytochrome a, b, c_1 oxidative activity.

GREEN: A point of clarification is in order with respect to the ratios. In the electron transfer chain there are fixed components that are linked one to the other within the four complexes and mobile components that are extractable and oscillate between complexes. All of the cytochromes except cytochrome c belong to the category of fixed components whereas cytochrome c belongs to the category of mobile components. The stoichiometry of the electron transfer chain that I am referring to applies only to the fixed components. In discussing variation in molecular proportions, we must consider only variations of one fixed component relative to another. I must ask the indulgence of the group for bringing up these specialized considerations because if the principle of precise stoichiometry does not apply to the electron transfer chain of a particular organism, then much of what I have said must be automatically disregarded.

SLONIMSKI: Not necessarily. It depends on whether the stress is on the homogeneity within the particle or between the particles. It may be intraparticulate or interparticulate.

GREEN: It would be difficult to encourage me, regardless of how you put it. Let us stay with stoichiometry.

SLONIMSKI: I cannot give the numbers in absolute terms. In terms of the order of magnitude——

GREEN: No, the relative ratios.

SLONIMSKI: Let us say the particle, which is a wild type, has a cytochrome a:b:c_1 ratio of 1:2:1. We can get a mutant with a 0:0:1 ratio. We have another with a 0:∞:0 ratio and can get one with a, let us say, 1:1:0 ratio. The 0 means not detectable.

GREEN: If I have understood your data correctly, the changes in ratio are predominantly, if not exclusively, expressions of deletions of particular cytochromes. That state of affairs would be acceptable to me. In a given complex a particular cytochrome would be missing, but the protein portion thereof or a modified protein could still be in the same place in the complex normally occupied by that cytochrome. Deletions do not disturb me. It is only the increases in the number of molecules of one component of a single electron transfer chain relative to the normal number of molecules for that or other components that would upset my equanimity.

SLONIMSKI: I must say I do not follow your argument very well. To you, the important ratio would be the ratio of a to b. Would this be the principal one?

GREEN: Let me put it this way. If normally there are 6 molecules of cytochrome a and 3 molecules of cytochrome b per chain, I would be in difficulty if in the mutant there were three molecules of both cytochrome a and cytochrome b per chain. If cytochrome a or b disappeared in the mutant, I could invoke the principle of deletion; but I cannot invoke that principle when the number of molecules of cytochrome a per chain drops from six to three. In the latter case we would be dealing with changes in the composition of the complex that contains cytochrome a.

SLONIMSKI: Within the complex?

GREEN: Changes in the ratio of fixed components when the numbers compared are whole numbers must mean that one or more of the individual complexes have a different chemical composition.

SLONIMSKI: I have given the reverse; I have given something that has a very great amount of a and no b.

GREEN: The absence of cytochrome a in a chain means deletion of a component or substitution by a hemeless protein equivalent. This type of variation is not what I would interpret to be a change in the stoichiometry of the chain. I have satisfied myself that it is the kind of variation that is permissible and compatible with the principle of stoichiometry.

SAGER: What are your criteria of what is permissible and what is not permissible?

GREEN: The principles of stoichiometry.

SAGER: I am sorry. It is really not clear. In terms of your picture of the mitochondrion, would you say it is permissible to have one complex missing and then have a system without heads?

GREEN: No, no, that is not what I mean! I can imagine that in these mutants the heme groups would not be synthesized. You would have the appropriate protein, but no heme group.

SLONIMSKI: No, the heme synthesis is in normal amount.

GREEN: When you say zero concentration of a particular cytochrome, what does that mean?

SLONIMSKI: It means no spectrum of a and no activity of cytochrome oxidase.

POLLARD: But you do not know there is no protein.

SLONIMSKI: I know because I have an antiserum. There is no cross-reacting material.

POLLARD: Is there physically no piece of material there where it would normally be seen?

SLONIMSKI: How can I detect it?

POLLARD: That is what we want you to realize.

SLONIMSKI: There is no a component when looked for either by physical properties, enzymatic properties, immunological properties, or by amino acid sequence. I cannot detect it by the criteria I am using.

POLLARD: That is a weakness in the statement.

DANIELLI: Since we are stuck on the exact relationship between Dr. Slonimski's data and Dr. Green's data, I would like to suggest that they sit together at lunch and find out whether the data they each have is compatible with a set of hypotheses and tell us after lunch.

We have time for one or two more points before we break for coffee.

McMULLEN: I must get something clear before we go very much further. Here I would like to tie Dr. Green down to something. When he says interfacial energies leave him cold——

GREEN: That is because I know nothing about them.

McMULLEN: You are not implying that you do not believe physical laws operate in biological systems?

GREEN: No, no.

McMULLEN: That is all right then.

FREMONT-SMITH: The anxiety is relieved.

GREEN: Thermodynamics is safe.

FOX: I would like to comment also on Dr. Green's statement about the self-organizing property. I do not find it particularly difficult to believe in this property, and partly I find this ease on the basis of a preprint that was circulated. It is possible to demonstrate for synthetic polymers the tendency for forming spontaneously units in which there is a crude kind of double

layer. On the basis of this, it does not seem to me to be so difficult to understand quite a few self-organizing properties in a system such as he describes. In fact, I wonder what he has that is more compelling than the concept of self-organizing macromolecules.

GREEN: Let me put it this way. I think it is a matter of opinion at the present moment. We cannot argue about that. The question really is, What degree of complexity can be arrived at by purely random processes?

FOX: That is also an assumption that they are random processes.

GREEN: We must presume that they are random.

FOX: Why?

GREEN: Well, then, if they are not random, what determines——

FOX: They are limited by their internal structure and function. This is the alternative presumption.

GREEN: To come back to the example I gave you where a large number of possibilities could be realized, what is——

FOX: That is presuming randomness, but evolution does not work that way. It is very limited in the extent of the potential diversity at any one step.

SLONIMSKI: This, I think, is a very important argument. This is exactly why Dr. Green has precise stoichiometric relations. They have been selected by evolution in the human heart or liver, and so on, while I am working to support artificial mutants which have not been selected by evolution. I am producing them under very special conditions, and their survival value is practically nil. They are immediately wiped out by any kind of competition with the wild-type yeast that has this precise stoichiometry which Dr. Green proposes.

So, what I am saying is that stoichiometry is not something that is chemically involved in the structure.

GREEN: Where Dr. Slonimski and I do not agree is on the question of how much change is permissible in a system so basic as the electron transfer chain. The mitochondrial system is built up of interlocking parts. If we radically change one part in respect to size by virtue of changing the number of protein molecules in that part, then every other part has to be changed if the system is to fit together. I believe that this is the primary reason why the basic structural pattern of the mitochondrial electron transfer chain is fundamentally the same in all forms of life.

Dr. Slonimski has studied a wide variety of yeast mutants with respect to their electron transfer components. In his laboratory Dr. Henry Mahler* compared the submitochondrial particles containing the electron transfer chain, in the wild type and in a mutant which apparently lacked most of the cytochromes. Dr. Mahler informed me that the particles from the two sources were virtually indistinguishable in size although the usual complement of cytochromes was missing in the particles derived from the mutant strain. The fact that the particle size did not decrease as the number of cytochromes in the chain was reduced surely points up that the chain is basically unaltered and that hemeless proteins substitute for the cytochromes in the various complexes of the chain where these cytochromes are normally present. The electron transfer chain of the mutant is admittedly defective, but the molecular architecture of the chain could still be normal in respect to the arrangement of complexes within the

*H. R. Mahler and B. Mackler, unpublished studies.

elementary particle and in respect to the arrangement of proteins within each of the four component complexes.

YCAS: I do not understand this last statement. If a, b, and c, are in particles and within each particle there is stoichiometry, in the collection of the particles stoichiometry must exist. Therefore, it is not necessary to isolate the particle for the purpose of discussing stoichiometry.

GREEN: But how do we know a particular cytochrome is in the particle or in X place?

YCAS: You defined it as being in that place.

GREEN: We can never know whether a particular cytochrome is present in a particle or outside the particle until we isolate the particle from the rest of the cell.

YCAS: When a whole cell or a mitochondrion preparation is isolated, do you or do you not find the same stoichiometry?

GREEN: We never do examine the whole cell. We always start with the mitochondrion. That is the beginning of our——

YCAS: This should give the answer at once as to whether there was self-ordering.

GREEN: It would be very difficult to do in the whole cell. This uncertainty of localization applies only to the whole cell, not to the isolated particle.

YCAS: A small particle does not have to be isolated. You would be satisfied if Dr. Slonimski did it on the isolated mitochondrion.

GREEN: Of course.

SLONIMSKI: This has been done.

DANIELLI: I think for the sake of the record it would be very useful if Dr. Green would write a statement explaining how he thinks this should be approached from the type of data he can handle and if Dr. Slonimski would do the same with his set of data. It is impossible to resolve the differences between you in the short time we have; therefore, I suggest we discontinue discussion on that at the moment.

HOFFMAN: In reference to Dr. Pittendrigh's comment about the internal milieu of the particle, it seems to me from what Dr. Green said about where each part is on his tripartite structure that we have to get the oxygen inside somehow to react with the particle IV. Therefore, I wonder what permease, or such, we have to get this oxygen inside—in whatever form we are going to get it inside.

GREEN: I think this would be largely a matter of diffusion.

HOFFMAN: Just molecular oxygen?

GREEN: Yes. The overall rate of oxidation of mitochondria is relatively low, compared to a yeast, for example; therefore, the concentration of oxygen may not be the limiting factor. Of course, I have not added an interesting complication. There is myoglobin associated very intimately with the mitochondrial membrane. Myoglobin forms a complex with the structural protein (ref. 41), and a considerable amount of myoglobin is localized in the membrane of the mitochondrion. Thus, there is one device by which we can increase the local concentration of oxygen. But I still think we have the problem of diffusion through the membrane.

SZENT-GYORGYI: I was very much interested in the statement that the conformational change in the actomyosin-like substance is connected with ion transport. The problem is that neither

actin nor myosin has an affinity to bivalent ions, but I do want to say that lately we found a protein that makes part of the contractile system, which has an enormous affinity for calcium and magnesium and is very easy to isolate. In one step we get a homogeneous solution. This will be found described in the Proceedings of the National Academy next month.

GREEN: I am delighted to hear this information from Dr. Szent-Gyorgyi. Our own picture of the collaboration between the actomyosin system of mitochondria and the elementary particle in ion translocation dictates that divalent ions should play a decisive role in triggering contraction. This is exciting news indeed.

(Midmorning break)

PITTENDRIGH: I felt very bad last night when I went home, because I thought my comments about theoretical biology did not really reflect what I think. I feel much more sympathetic to it than I indicated. I felt the least I could do was to make a constructive effort, which I have had only a few minutes to put together. I thought we ought to be concerned with a model for a minimum theoretical biologist rather than a model for a minimum cell. The beginnings of this follow very much Dr. Morowitz' paper.

It is clear that we need some DNA; we need a ribosome—one, but I could not get to his 45 enzymes. I can see 23. We need a Kornberg enzyme, we need a Morowitz enzyme, and we need 20 for the PRNA; that gives me 23.

Trying to be quantitative again, the question is, How many cistrons are in a DNA? I can see several constituents that we need. We need, principally, to identify the problemase, perceptase, imaginase, and a criticase. We need an easily inducible provisional simplificationase. I do not know whether the following should be inducible at all, having in mind a conversationalase, a nominalase, and a verbalase, which we have been showing conspicuously in the last two days. I decided to eliminate altogether an overwhelmase. I assure you this is a provisional start, and I think it would be a worthwhile undertaking to enlarge it, to find out exactly what theoretical biology is all about. Thank you.

QUIMBY: Dr. Pittendrigh, do you still think this is constructive?

PITTENDRIGH: Oh, yes.

DANIELLI: We managed to deal with about 1 percent of the problems, if that, under the heading we had for the first session this morning. I think we must now move on to the second major topic under organelles; that is to say, the specificity of organelles and their replication. I would like to make one or two opening remarks before asking Dr. Ycas to say something further.

We have heard quite a lot about spontaneous versus nonspontaneous assembly, and I think we have to consider first the alternatives to spontaneous assembly and, secondly, the possibility that the cell deliberately avoids systems which will assemble spontaneously in order to have them under control.

One of the problems we are confronted with in the organelle situation is that there are some organelles which seem to be cytoplasmically inherited in certain respects. For example, we sometimes find we need one of an organelle before we can get two, and if we have none in a cell there may be present all the nuclear genes necessary and yet no organelle is formed. The question arises Why? There are a number of possible answers to that, of course.

Then, there is the question of the alternatives to spontaneous assembly, and I imagine we shall succeed in drawing up quite a list of these. I am going to put forward two which I suspect are, in fact, quite well known. One is that, even in the case of the so-called cytoplasmic inheritance, the system with which we are dealing is really still based on nucleic acid and the

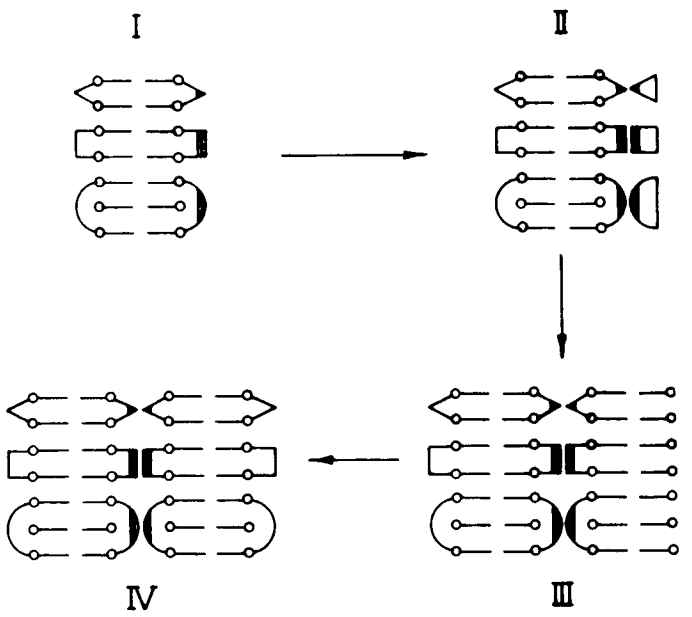

Figure 18.—Linear polymers interacting to form a two-dimensional network.

nucleic acid produces linear polymers. If we assume now that these linear polymers are capable of being arranged so as to interact, so as to form a two-dimensional network (which will involve specific interactions between sites on the polymers so as to give two-dimensional networks), then we can readily see if each intersection in the net is now a specific absorbing center for a particular macromolecule (fig. 18). Thus we could get the assembly of two-dimensional structures without anything other than the coding of linear polymers in the first instance. This does not require that the things totally fall together, because I think we can see that it would be possible for the messengers for synthesis of these longitudinal polymers to be assembled in a particular part of a cell—we are not prepared to specify that mechanism immediately—and there is no reason why we have to specify spontaneity in a system like this.

Alternatively, we do not need to assemble all the molecules for such a structure in this one place. All we need to assemble, it seems to me, is the basic linear polymers which will give a two-dimensional network. Then we could rely upon spontaneous assembly for the second layer molecules.

The second hypothesis is based on the idea that the individual membranes of the organelles act as supertemplates. This means that each macromolecule in the original membrane is represented by an identical macromolecule in the derived membrane, and the lipid bilayer is formed as part of the process of assembling macromolecules. This process can formally be divided into a number of discrete stages, as indicated in figure 19. The feasibility of such a scheme depends upon the selective interaction between macromolecules across the thickness of a bilayer. This may be based either upon the charge-dipole distributions giving rise to a selective field or to the inducing of long-range order effects over a distance of about 100 angstroms.

GREEN: Suppose that there is the additional requirement of setting up two layers of particles separated by a space in which are dissolved a set of soluble small coenzyme molecules. These small molecules have to be introduced in the space under conditions where they cannot leak out. Then in addition the layers of particles with the intervening space have to be wrapped around a matrix core.

DANIELLI: Actually, there are three problems besides what I have lined up here. To these I have no solution at the moment, but the first is the one you mentioned—how to fill up the space between the two membranes of mitochondria.

MOROWITZ: I want to make a comment relative to what you said initially concerning where a great part of the impetus comes from for believing these structures are crystallizing out. You do not like crystallizing—why these things are forming spontaneously? I refer to the

experiment of White and Anfinsen on ribonuclease (ref. 57). The secondary structure of ribonuclease looks terribly complicated and difficult to assemble. Apparently it has to be done in a rather specific way. In their experiment, they broke the four sulfur-sulfur linkages leading to a long-chain molecule which is functionally inactive as a ribonuclease. The breakage was by reduction of sulfur-sulfur bonds.

In their second experiment they gently bubbled oxygen through a solution of reduced ribonuclease in order to oxidize the sulfur-sulfur bonds; the right ones join up again, and the structure and enzymatic activity is restored.

GREEN: You mean they have an enzyme for that purpose?

MOROWITZ: This is done without an enzyme. The reforming into the exact structure is done by bubbling oxygen through the solution. This is a case again which, before the experiment, might have looked as though a hopelessly complicated sort of secondary structure was being made, but, in fact, it does come about in a very simple way.

DANIELLI: Does anyone want to bubble some criticism through this?

GREEN: I do want to repeat that they have, in fact, found that the process proceeds normally under the influence of a specific enzyme which they have isolated and purified. I am not sure whether the spontaneous reoxidation does lead to exactly the same product as the enzymatically reoxidized component.

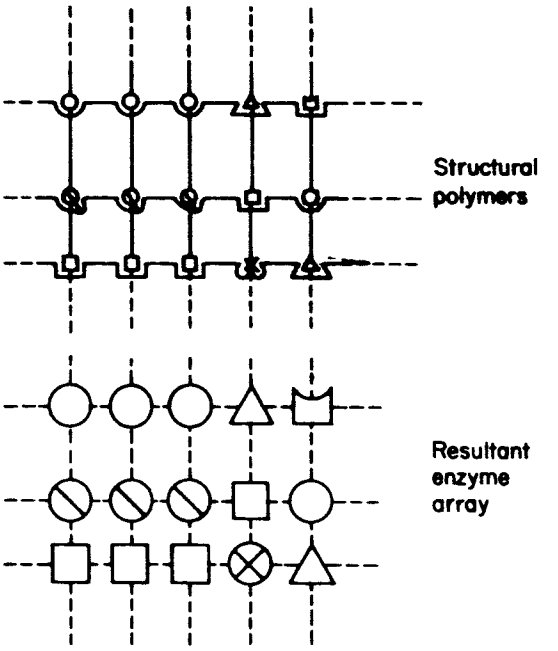

Figure 19.—Hypothesis based on individual membranes of organelles acting as supertemplates. Each macromolecule in the original membrane is represented by an identical macromolecule in the derived membrane. The lipid bilayer is formed as part of the process of assembling macromolecules. This process can formally be divided into a number of discrete stages, as indicated.

FOX: Does it not lead, however, to an enzymatically active product—the same sensitive criterion you are invoking?

GREEN: That is just the criterion I was referring to. Does the spontaneous process lead to an active enzyme?

LEVINS: Looking at the problem a little more abstractly, the question of spontaneous formation of structure seems to be the following. We have a region of space and within each small region are different concentrations of various substances so that there is a set of differential equations describing the changes at each point.

Since this is taking place over all the points in space, we have a set of partial differential equations. We are asking the question, Is the final steady state dependent on the initial conditions? We know that, if we change the composition of the cell, we can get different end states. We are asking the question—Keeping the same average composition but just changing the structure, will the system give us a different steady state? How many different steady states are possible, depending on initial conditions only, in structural change?

This would be quite sensitive to the kind of differential equations you have, and I think that it would be amenable to analysis in the field of qualitative differential equations—simply the question of what kinds of loops and control mechanisms and interactions will increase the likelihood of having multiple steady states as distinguished from a single steady state.

LEIGH: Is there a branch of qualitative analysis of partial differential equations analogous to that of ordinary differential equations which would permit what you wish to do?

LEVINS: I do not know. The only thing I can think of is the Nemytskii-Stepanov group at the University of Moscow (ref. 58).

LEIGH: Most of the Russian work (ref. 58) that I can remember is ordinary differential equations. I cannot name the mathematical reasons why there would be difficulties in carrying it over to the partial differential equations, but I do remember that many of the most interesting analyses that come out of this would be in terms of things approaching limit cycles in a very precise sense. And talking about limit cycles in things involving more than two variables is very hard work.

SLONIMSKI: I would like to suggest that it is possible for a theoretical biologist to try to put together models which are amenable for the more precise analysis of the phenomenon of intragenic and intrallelic complementation in vitro of enzymatic activity. The phenomenon is very simple. We take an enzyme, we make a mutant that knocks out completely the enzyme activity, we take another mutant that knocks out the same enzyme activity, then we make an extract of both mutants which have no enzyme activity, and then we mix them and obtain enzyme activity.

All these things are probably due to interactions between chains, and it is not clear whether the interactions between chains are in terms of protein-amino acid sequence or whether the interactions are between slightly different chains of two similar polymers. But, experimentally, the system is very easy to analyze and theoretically, while I know of only one attempt that has given a rather complicated three-dimensional structure, there may be completely different ways of trying to analyze and to produce a model. The model which has been proposed was a helix. You may have seen news of it a few months ago.

GREEN: Such a program would in effect involve the emergence of new activities by new combinations of chains each of which is defective.

SLONIMSKI: This is something I thought could be done. Experimentally, it is easy because a few hundred fermentations can be made in a few weeks.

ENGELBERG: Dr. Morowitz, in the experiment of White and Anfinsen (ref. 57) that you cited, is there evidence that all the sulfur-sulfur bonds were reformed correctly or was it just a proportion?

MOROWITZ: I do not want to start quoting the paper in detail because I do not wish to trust my memory. For instance, if they operate at too high a concentration of ribonuclease they get an insoluble precipitate because all they do is cross-link chains. I think they reported as much as 60 percent of the activity restored in some of the experiments. It was around that order of magnitude, and I think it is also clear that, doing it this way, some wrong sulfur-sulfur bonds would probably be formed. What is more significant, though, is that even doing it in what is a quite violent way for the process, they end up with about the right thing.

ENGELBERG: This experiment implies that the structure of an enzyme depends on the sulfur-sulfur bond, but that, if we want to get the same molecule, we still have to associate the right sulfur bonds.

MOROWITZ: No, no. What this experiment is saying is that a good deal of secondary structure is implicit in primary structure and that this kind of principle which we see in the enzyme ribonuclease is also seen in the tabacco mosaic virus and in any virus assembly. Virus assembly of the large animal viruses may be a more direct example. These results indicate the possibility of obtaining rather elaborate structures without having specific pieces of equipment in the cell whose function it is to assemble these structures. These structures can assemble because this is the most probable chain of events on collision processes.

WATTS-TOBIN: I only wanted to say I think there are two problems. First, if we have many mixed-up components, will they sort themselves out into a regular structure? Second, if we are actually synthesizing things—for example, proteins—will they, while they are being synthesized, fold up in the right way?

These are really two quite different problems. In the case of protein synthesis, for example, there is a very complicated mechanism indeed, the need for most of which is still not at all clear; but it may be that it is connected with insuring that the nascent protein does fold up in the right way as it is produced. The extrapolation from that to something very much larger, such as these membranes, is going even further.

FOX: But the principle is there.

WOESE: To comment further on Dr. Watts-Tobin's point, certainly there is ample evidence on the cellular level at least that a "sorting out" can occur. Embryologists can disrupt, for example, a developing egg into a random mixture of the component cells. In time the cells of a particular type (for example, ectoderm or endoderm) will find one another and clump together, given proper environment.

WATTS-TOBIN: Does this really imply that the cell is in such a sort of pseudostable steady state that, no matter how it is stirred up, if it is left long enough it will get back, under the same external conditions, to its original structure? Are you really suggesting that?

DANIELLI: I think before we continue, it might be useful to come back to a point made by Dr. Levins. He raised the point concerning whether, if we begin with a cell of a given composition, we have the possibility of ending up with two different populations of organelles. This happens in a general way during differentiation. I think this is very relevant to consider, but it is also very difficult to achieve experimentally, except in a differentiating system.

There is a further point of interest. In the case of the amoeba that we have been investigating, we find some characteristics which are wholly controlled by the nucleus and which we think are probably concerned with the internal structure of the macromolecules where the character can be expressed by the activity of the individual macromolecules. We find no cases whatever where cytoplasmic inheritance is the only factor concerned in determining a character, and wherever cytoplasmic inheritance turns up we always get control by a nuclear factor as well.

An interesting thing here is that wherever cytoplasmic inheritance turns up, it seems to be with arrays of different types of macromolecules organized into high-level structures. We never get cytoplasmic inheritance with a low level of organization of structures.

PITTENDRIGH: What do you mean by "low level," Dr. Danielli?

DANIELLI: Take the two simple examples. Hemoglobin can do its job without being organized into a structure. Similarly, the antigenic property of the surface antigens of cells does not depend upon there being neighbor molecules. When we look at these characteristics, we do not get any evidence of cytoplasmic control.

On the other hand, if we go to something such as the shape an organism assumes when it is moving, which is the property of a very large array of macromolecules organized in a

specific way, then we find strong evidence of cytoplasmic control as well as of a nuclear control.

LEVINS: I think we are limited by the limits of cross compatibility to the study of similar organisms. There is reason to believe that the small adaptive differences between populations of the same species are determined by nuclear genes primarily. When we get to groups that are more taxonomically remote, the experiment cannot be done, and those are the places where we would expect permanent structural cytoplasmic differences to be more important.

SAGER: There is a growing body of evidence, both from genetic experiments and from biochemical experiments, for the existence of an extensive amount of genetic information which is not carried on chromosomes. There is so much evidence, in such a variety of organisms, that I cannot take your time now to review it. In Chlamydomonas, which is a single-celled green alga with which we have been working, we have found a mutagen which is very effective for inducing mutations in the nonchromosomal (NC) system. The mutagen is streptomycin; its mode of action is very different from that of mutagens for chromosomal genes.

The fact that streptomycin is effective suggests that the material which is being mutated may be nucleic acid, but the evidence is indirect, being based on the in vitro reactivity of streptomycin with nucleic acids. Many of the phenotypes affected by mutations in the NC system are phenotypes involving organelles; for example, chloroplasts and mitochondria are great targets for mutations in the NC system. This does not prove that the NC genetic information is located in these organelles, but it does show that NC genes are important in organelle formation. There is as yet no decisive experiment relating the existence of primary genetic information which is nonchromosomal to any particular structure.

However, there is biochemical evidence for the existence of DNA in chloroplasts of many organisms. We have recently published evidence for the presence of DNA in the chloroplasts of Chlamydomonas, and there is increasingly good evidence, both published and unpublished, that there probably is DNA associated with the chloroplasts of all higher plants. However, no experiments as yet provide any evidence about the function of this DNA. It simply says that chloroplasts contain DNA.

There are also electron microscope studies that show the presence of fibers in mitochondria as seen in the electron microscope, which can be removed by prior treatment with deoxyribonuclease. This work provides one line of evidence that there may be some DNA in mitochondria.

If organelle DNA is functional, presumably it would act in a manner similar to that of chromosomal DNA by determining the specificity and the rate of synthesis of particular proteins. We may then ask whether additional classes of genetic material other than DNA may also exist.

There is as yet no reason to assume that all genetic material is DNA or RNA. Are these one-dimensional molecules sufficient for determining two-dimensional membranes and organelles? This kind of genetic information may be found only in higher organisms. I would call a higher organism anything with more than one chromosome, a nucleus, and a nuclear membrane. I chose these criteria because cells of this sort, whether they are single-celled organisms or cells of multicellular organisms, have the same kind of subcellular organization of the cytoplasm, the same kind of organelles. These similarities, I think, are the ones of most importance to us in trying to understand the control of subcellular organization. Thus, for these purposes, I like to call them all higher organisms.

As far as these organisms are concerned, there may be a subdivision of genetic information that has never been appreciated before into chromosomal and extrachromosomal sectors. Nonchromosomal heredity has been difficult to study. There were no methods of regularly obtaining mutants so there was very little material to work with. Also, because nonchromosomal

genes show maternal inheritance, there were few opportunities to see segregation occurring in these populations. These methodological limitations are now being surmounted, and it is beginning to appear that NC genes represent an important part of the total genetic information in all organisms.

The question remains open whether this second genetic system we have mutated with streptomycin may also be a nucleic acid system and may be very similar in the things that it does to chromosomal genes; that is, in coding for the primary structure of proteins and in regulating the rate of protein synthesis. There may be yet a third system, which is a system of the sort Dr. Danielli has considered for some time and which many of us have been interested in; that is, some kind of a macromolecular template in which two-dimensional structures of the same kind, and in which there is some kind of specificity, a specificity having to do with the relation of macromolecules to each other.

I do not know of a single case in which it has been rigorously established that the loss of an organelle is responsible for failure of the organelle to reappear in the population.

GREEN: I wonder if I could inject a comment at this point. The mitochondrion that we have been working with can be prepared on such a scale that it is possible to answer very directly certain questions. For example, is there any nucleic acid in the mitochondrion? This is a problem that can be tackled by direct isolation. This we have carried out in collaboration with Dr. Rammler. * We can say unambiguously that there is no significant amount of nucleic acid in the mitochondrion of the beef heart, that there is, in fact, a polynucleotide containing about 10 base units. Obviously this polynucleotide could have nothing to do with replication of the mitochondrion.

McMULLEN: Is this deoxy or ribose?

GREEN: It is ribose and its chain length is such that we suspect it plays some role as yet undefined in one of the enzymatic processes.

If we accept the evidence at face value, it means that, at least in one membrane system, we can say the chances of this postulated self-replication within the mitochondrion are nil. If we assume that the membrane systems are laid down in much the same way as the mitochondrion, it would, I think, throw considerable doubt on the idea that any other membrane system would be self-replicating. From the similarities in the structure of membranes generally, we would expect that the mechanism of replication would be much the same and would probably be external.

BAUTZ: To set the record straight, I think for me the question is this: Is there any evidence for a system of replication that does not involve nucleic acid? Could we approach it this way? All of Dr. Sager's evidence points to the fact that nucleic acid is involved in the information, and I do not think we should now discuss whether it is DNA or RNA because, in principle, they both can function.

ENGELBERG: I am just wondering if there is a basic theoretical problem involved. All of the mechanisms mentioned here appear to be in the realm of possibility so that the question is an experimental one as to whether certain organelles reproduce this way or that. I do not think we can settle this by having certain leanings as to whether the process is random or not. More information is necessary to settle the question.

DANIELLI: I think that is a particularly valid point of view. I think the value to the community in discussing this from a theoretical point of view is that we have not thought sufficiently about

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*D. Rammler, unpublished studies.

such systems, and the experiments are very difficult to set up—very difficult, indeed, to be absolutely critical.

Therefore, what we want to assemble is as many working hypotheses as possible concerning how such replications of structure could be brought about. Then we are in a much better position to operate economically as an experimentalist. If we have only one hypothesis, we, so to speak, set up an experiment to try to find out whether that one is right or wrong; but if we have half a dozen hypotheses, it is possible to set up one experiment that will bear on several of these hypotheses simultaneously. Therefore, I think the question of different ways in which one-dimensional or two-dimensional structures can be assembled is a legitimate theoretical study because this not only has an interest in itself but also is a guide to experimentation.

McMULLEN: I would just suggest that if P and Q on Dr. Danielli's diagram are Dr. Green's Chancellor cases for low molecular weight substances and A and B are syntheases or polymerases, then is not Dr. Danielli's little problem more or less solved naively in that the low molecular weight substances will go through and under osmotic or other translocation forces and build up in the interior filling in the gap with the biological fluid that Dr. Green wants to fit in there?

GREEN: I would like to mention some experiments which offer a hope of resolving the question experimentally. During spermatogenesis the mitochondria is disassembled into its component parts; in the sperm head these parts are now woven together like rope; and then after fertilization the mitochondrion is reassembled. Andre (ref. 59) in France has done a beautiful piece of work on this series of transformations. My contention is that it should be possible now to disentangle the steps by which the mitochondrion can be resolved into its parts and then reconstructed.

This is a very unusual occurrence for the mitochondrion to be dismembered into its parts: it happens only during spermatogenesis. The reassembly of the mitochondrion offers a magnificent experimental opportunity to resolve this very question.

BAUTZ: I wonder whether we could take the question of whether there is one mode of replication that brings results from an evolutionary standpoint. I would like to put the question this way: "If there existed two modes of replication, would one outgrow the other?" I would say that, for me, extrachromosomal inheritance and intrachromosomal inheritance are the same mode of replication. This is no different system of replication.

LEVINS: The evolutionary consequences are quite different.

SLONIMSKI: If I understand correctly, Dr. Bautz asked whether there is a different mode of replication in the nucleic replication.

SAGER: If the entire problem of cell organization can be solved by synthesizing the right components in the right place and at the right time, there is no need for anything beyond nucleic acids as primary genetic information. Therefore, the question really is whether nucleic acid type of information is sufficient to make a cell. I think that this is a key question for theoretical biologists, and it is a worthwhile question because the probability is high that it will be answered in the negative.

PITTENDRIGH: Encouraged by Dr. Fremont-Smith's words of advice at the beginning, I am going to ask a question which makes me blush slightly because I really ought to have followed the literature on this, but I have not. Do we know at the moment when, in antibody formation, the nucleic acids are involved, other than that a protein is made? Is a nucleic acid involved in specifying the specific surface of the antibody which reacts with an antigen?

Otherwise, we go all the way back to Sterling Emerson's model of the 1940's for gene replication before the Watson-Crick era began, and it seems to me that since the Watson-Crick

model so obviously offered the solution, Sterling Emerson's really splendid idea has been buried. That is to say, if we can make a specific surface with a protein and it does not involve nucleic acids to specify the individual surface, do we have a basis for another mechanism of specific surface replication?

I therefore ask the question as a simple matter of fact whether or not at the present time we have any evidence that RNA is needed for the specification of the surface of the antibody.

ATWOOD: There is an answer to this: it is not known whether RNA is needed to specify the binding site for antigen, but it is known that other parts of the antibody molecule are specified by ordinary genes—the allotype portions.

PITTENDRIGH: How is the specificity of the binding site established?

ATWOOD: It is not known.

DANIELLI: Where do we go from here?

WATTS-TOBIN: Could I ask, Dr. Danielli, from complete ignorance just how much interaction we would expect to get through relatively nonpolar membranes and what sort of energies we might expect to get between two proteins, one on one side and one on the other.

DANIELLI: I do not know. I have not done anything about this. It is only just this last month or two that I have begun to think about these membranes again, and we have a number of problems—of which this is one, involving macromolecular interactions—and also the general problem of setting up an equation for such lipid layers. As far as I know, none of these problems has actually been undertaken yet, even to defining the order of magnitude of the forces, and so forth. You probably know more than I do about this, Dr. Pollard.

POLLARD: I cannot answer this, but even with 6 angstroms there should not be much force. We have been decreasing it from 20 to 6 for the dielectric constants, and it is still not easy. A structural arrangement would have to be involved in the main force. There is further resolution at short range.

WATTS-TOBIN: What sort of forces are you thinking of?

POLLARD: Any physical force.

WATTS-TOBIN: This does not apply to electrostatic forces.

POLLARD: There are really practically no free electrostatic forces in any materials we know of. If there are no ions present and the membrane is charged, then there is a lot of excitment.

DANIELLI: That is just the situation.

WATTS-TOBIN: Just what is the situation?

DANIELLI: That the molecules you are interested in are charged.

WATTS-TOBIN: That is what I meant. The question is, surely, whether the ionic strength of the environment is so high as to screen this all out.

DANIELLI: It cannot do so because the ions are not sufficiently solid in the lipid layers. The ionic strength is practically zero. In the aqueous phase, yes.

POLLARD: Yes, or if we just have bonds alone, we are in fine shape—lots of juice.

FOX: I would like to ask at this point if we are faced with conflicting points of view—relatively direct genic control of morphology on the one hand and self-assembling properties of protein molecules on the other. Is this a conflict, or is it not? It appears to me it is not, but if

there is any reason to believe it is, I think there is a dualistic point of view that reconciles the problem. This is simply the point of view that nucleic acids specify amino acid sequence, whereas morphology is determined by the conformation resulting from the sequence. The genic control is thus indirect.

DANIELLI: Years ago this would have been regarded, I think, as a situation in which there was conflict, but it seems to me that discussions in the last 5 to 10 years have gradually worked up to a recognition of the fact that there are quite a number of very real alternatives here which can only be resolved by experiment.

FOX: That is the way it seems to me. Preferably, control experiments in systems in which genes are absent should be included when that is possible.

DANIELLI: You may be right.

ENGELBERG: I was wondering if we could come back for a moment to the problem you raised before about the way to fill the space between the two layers with small molecules. In view of the fact that in three-dimensional space only one-dimensional and two-dimensional objects have a surface, would we not have exactly the same problem whether it is random assembly or whether it is directed assembly? In other words, we cannot have a directed assembly of a three-dimensional object with one blow; we always have to pass through the stages of two-dimensional assembly.

GREEN: It would have to be sequential. It could not be done in one fell swoop.

ENGELBERG: Yes, and it could then very well be sequential if it is spontaneous, if one component is the negative of the other.

SAGER: In the assembly of a virus particle there is no two-dimensional stage because the protein subunits simply fold around the nucleic acid.

POLLARD: Is that not really one strand? It is a long thing. Admittedly, it is coiled, but does it not essentially go along the length and, therefore——

SAGER: But they are also spherical.

POLLARD: Are they spherical or are they actually made by going along and then finally convoluting their way to conclusion at the sphere? They are not made this way; they are made in a radial wave.

POLLARD: I think they are aggregated along the line and coil up.

ENGELBERG: Technically speaking, it is a one-dimensional structure embedded in a three-dimensional space.

PITTENDRIGH: I wanted to ask a question that does not immediately bear on the present discussion but I think it relates somewhat. How do the cytochromes look on the bacterial plasma membrane? Do we see these strange buckings that are seen on the mitochondrial wall? I take it that the cytochromes are, in fact, on the plasma membrane of the bacterial cell.

GREEN: I would imagine so from what has been described, but let me put it this way. We have isolated from Azotobacter a particle containing the electron transfer chain. We have carried out a complete chemical analysis of this particle. The gross chemical composition of the Azotobacter particle is indistinguishable from that of the corresponding particles from beef heart mitochondria. The stoichiometry of components is the same as is the lipid content. Thus in all qualitative respects we find nothing to distinguish the bacterial system from the pig heart or beef heart system. For that reason, we are inclined to suspect that the basic

geometry, stoichiometry, and chemical composition of the electron transfer chain in mitochondria from any source is going to turn out to be the same. There have been similar investigations in other types of mitochondria, and from all accounts where chemical work has been done, the basic characteristics are the same.

You are asking whether we would see these elementary particles in Azotobacter. That has yet to be done. The use of phosphotungstate as a fixing agent, I would think, would be mandatory for such electron micrograph studies.

PITTENDRIGH: If I understood the exchange before the coffee break, in some cases you thought that the stoichiometry of the situation demanded the structural arrangement.

GREEN: Yes.

PITTENDRIGH: Therefore, you really have a very strong prediction here which somebody ought to be testing.

DANIELLI: This is right. It should be of value to make that test.

GREEN: The mitochondria of Neurospora have these elementary particles. Stoeckenius (ref. 19), I think, did the electron microscopy.

PITTENDRIGH: These are mitochondria, but I am asking, when about the cytochrome system——

GREEN: He is referring to the cytochrome system in Neurospora.

PITTENDRIGH: But my point is, when we get the same macromolecules into a phytogenetically different organization, if the stoichiometry is the real consideration, then we ought to get the same structure in bacteria.

GREEN: That is a very good and sound prediction. The fact is, it has not been done in enough cases. All membrane systems, when carefully examined by electron microscopy, do show repeating particles, but not necessarily the particles containing the electron transfer chain. The red blood corpuscle membrane shows evidence of repeating particles. In fact, Stoeckenius has examined an extensive series of membrane by the negative staining technique, and he has evidence of particles in every one of them. This may be a regular feature of membrane systems—the association of macromolecular assemblies with the layers.

POLLARD: If I might, Dr. McMullen has pricked my conscience and he has been saying we should discuss the question as to whether we can synthesize a cell de novo in such a way that it looks like a cell as we see it today, or whether we have to go a long way back and de novo synthesize a cell that looks like it used to be a billion years ago.

ATWOOD: Or neither one of those.

POLLARD: We have a quiet half hour, and we are well stimulated because we are hungry. It would be a good idea if Dr. McMullen would talk about this a little bit.

GRENNELL: Could I ask one question about some of the things we have been talking about before that?

DANIELLI: Perhaps we did not get the message across but, for various reasons, we felt we had to telescope the meeting into 2 days and omit the session tomorrow. Therefore, the likelihood of the evening session being, as was originally planned, on the method of constructing a cell is remote, unless there is absolutely no discussion on the ecological side. We plan to begin the ecological discussion immediately after the afternoon coffee break and it may well take up the rest of the day.

Dr. McMullen, would you like to go ahead?

McMULLEN: If I am not anticipating some of Dr. Fox's work, I will take a few minutes.

DANIELLI: That will be all right; Dr. Fox is speaking immediately after lunch.

McMULLEN: I just want to initiate some discussion and I will not take very long. I would like to go back to the question of the primeval soup to consider the various possibilities for the evolution of specificity, more or less on the lines Oparin has suggested, because I think we should, somewhere in this conference, discuss this kind of thing.

If we have in primeval conditions this pond water, this primeval soup, with energy beating down on it causing evaporation and concentration of simple substances or combinations of substances which have arrived by prior concentration in the atmosphere as things cooled down, the elementary atoms coalesce (I do not want to go further back than the elementary atom!) and at some stage the molecular weight of the condensed products will become so great that precipitation in the form of water and other substances will occur.

We have, therefore, low molecular weight materials in our aqueous solution which can be of extremely high concentration as evaporation proceeds in isolated aqueous pockets. Under these conditions, physically, we can obtain phase separations.

If we accept that, if we accept the possibility of tactoids or structures like coacervates, et cetera, where one phase is separated from another purely by concentration, then it has been demonstrated that with such an interphase we can attain orientation of molecules of almost any kind, that the greater the asymmetry of the molecule the more chance there is of orientation. Thus if we can visualize obtaining in such coacervates absorption at their interphases of molecules having a certain complexity and therefore a certain specificity, then with these simple systems one can demonstrate phenomena such as activated diffusion or activated transport from one phase to the next.

We can further envisage the possibility of osmotic pressure forces existing and this coacervate increasing in size to such a point where these interfacial and interior forces are in disequilibrium; the coacervate replicates in the sense of a rather loose interpretation of the term "replication" (I mean multiplies), and it will split into a system where these forces are again in a dynamic equilibrium. Continuation of this process will give multiplication of these elementary type structures. In one of these structures, we have a number of substances in which continuous changes, interactions, are taking place, and certain specific molecules are absorbed around the interphase, these substances being essential for the stability of that structure.

This picture gives the idea that the only kind of structure in this system, which can be called an ordered structure, is this interphase. We can suggest, theoretically on the basis of Dr. Danielli's picture, how we can build up an identical structure by absorption which is, in fact, replication of the essential units of this structure, and only the essential units will be replicated by virtue of the fact that they are essential for the stability and subdivision or multiplication of this entity.

Any other substance which was initially in the body when it multiplies ad infinitum over a period of so many years, unless the substance is in effect replicated in succeeding daughter cells, will die out of the system. The final result umpteen years later, with materials selected in this body, by virtue of their necessity. We can then call these specific enzymes, if you like, not because something like DNA has given them a particular subunit sequence but because of their initial structure in the primary body, being essential for the continuance of this body.

The possibility of this happening is impossible to calculate, but——

ATWOOD: Zero.

McMULLEN: No.

ATWOOD: That is my figure—zero.

McMULLEN: The possibility of a molecule, protein, or DNA forming spontaneously is near zero, but this is not what I am talking about.

ATWOOD: You are going to tell us how it replicates itself?

McMULLEN: No, I am hoping you will suggest——

POLLARD: What is the meaning of the word "essential," because it is perfectly possible for it just not to exist. Why does it have to exist? The word "essential" says it must exist.

McMULLEN: If we can envisage many types of coacervate structure arriving initially, only one or two of which will be sufficiently stable to exist for any length of time; this means that if they exist long enough they have a chance of complicating their structure to increase that resistance, to enable them to exist for a greater length of time. Those which cannot do this will not exist, but those that are structurally arranged, through chance, to exist longer than others will do so. And at some stage where they then subdivide as described earlier, they have produced more of themselves, which have a better chance of existing than those which were not structurally organized to enable them to divide or to divide so successfully. In other words, this is just selection by environmental conditions of nonliving bodies—chemicals, if you like, or agglomerations of chemicals.

This is the kind of thing that various people have talked about. I am just attempting to summarize it, and I really do not know enough about it to make a very good and accurate picture, but it struck me that at least this type of consideration of the evolution of cellular bodies or structural replicating entities like these "molecular machines" is theoretical biology and should be considered at least for half an hour in a meeting of this nature.

I wonder if anyone would care to comment.

DANIELLI: If I understand it correctly, you are going to give us an example of a system that is not totally dissimilar to this. Is that right, Dr. Fox, or did I misunderstand?

FOX: No, that is correct.

POLLARD: I wonder if Dr. Roberts would want to comment on a completely different method that he has talked about in which he actually tried to evolve the DNA-RNA protein systems from research.

ROBERTS: For just a minute. It would seem that if we were content with a very inefficient enzyme, which we might get by specifying 6 amino acids out of a chain of 200, it might have an efficiency of one compared to present-day thousands. But it still might be a very fine thing in the early days. When we calculate the probability of making the template for that particular enzyme by chance, it is reasonably high. If we synthesize a milligram of random nucleic acid, it will contain a very large number of templates for that particular enzyme. If we are content with other enzymes of that same order of efficiency, a milligram will contain templates for all of those enzymes, too. In fact, if we go down to a 10-micron sphere, we find that we have roughly 10 templates for any particular enzyme that we would like to specify.

If those do include a primitive Kornberg enzyme and a primitive polymerase and other essential enzymes, we have a chance of getting started. The particles which were lucky and had their full complement would grow faster than ones which are statistically unlikely in that they have 1 instead of 10. Let us start with RNA and advance to DNA later.

DANIELLI: I think the possibility of synthesizing models of the active centers of enzymes is rapidly becoming realistic and might even enable us to set up directed experiments of the nature you are suggesting.

ATWOOD: The difference between this way of looking at it and Oparin's way (ref. 60) of looking at it is that Dr. Roberts begins with the manner in which one sequence specifies another and in which the specifying sequence will, itself, be duplicated. The necessity for point-to-point duplication at the molecular level apparently did not occur to Oparin (ref. 60). I meant to recognize this point in saying that an enzyme does not derive any probability of being perpetuated from the mere fact that its activity increases the size of the coacervate in which it first appeared. It certainly would not recur unless there is in this system a mechanism that specifies its sequence and duplicates the specifying agent.

McMULLEN: Yes; I am suggesting that the membrane interphase structure there——

ATWOOD: To put it shortly, Oparin (ref. 60) did not understand genetics and, therefore, his speculations cannot be taken seriously.

McMULLEN: I do not think you can say that.

LEVIN: Perhaps he was dealing with a pregenetic swirl!

ATWOOD: Intermediary metabolism has a pregenetic history, but life, by definition, does not have.

TOTTER: I have wanted to call attention in connection with Dr. Roberts' words that it apparently takes about 18 milligrams of DNA to specify all the individuals in the human race, which is a good figure to think of in connection with the tons of material that are available from the kind of things Dr. McMullen was talking about.

CROW: Is it worth trying to distinguish what we mean by genetic in this context?

SLONIMSKI: That is what I wanted to say.

CROW: What I think is that by "genetic" you mean some element that has the property of copying itself and, if it makes a mistake, of copying the mistake.

SLONIMSKI: This is a very broad notion of genetics. What I was always told when I started genetics was that genetics begins when there is a mutant. If we do not have a mutant, we do not have genetics.

CROW: That is what I am saying, too.

DANIELLI: That is not true.

CROW: Your definition of the mutant is that it copies itself in the mutant form, not in the original form.

DANIELLI: Right.

SLONIMSKI: But we can invent a system that will help the genetic combination of information, without mutation and without recombination, which will still be genetic, but it will not be amenable to genetic analysis.

ATWOOD: What would you make it out of?

SLONIMSKI: Suppose we have a DNA, or RNA, or any kind of nucleic acid that perpetuates itself. It has the Kornberg enzyme, but it never mutates.

ATWOOD: You cannot think of anything we can make it out of that would not have to mutate sometime?

SLONIMSKI: No.

ATWOOD: Unless we run at absolute zero.

SLONIMSKI: If we consider a mutation something we can cross with it—Suppose we had a six-element unit before; the six-element unit already had the capacity of duplicating, but the three-element unit did not have the capacity of duplicating. If it does not have the capacity of duplicating, we cannot cross it. We cannot cross a three-element unit with a six-element unit because the only one that duplicates is the six-element one.

I am not sure whether I am clear.

ATWOOD: I was only saying that if we are going to make organisms out of matter, then we will have a genetics of mutation, at least.

CROW: Historically, the genetics of mutation is more primary.

SLONIMSKI: Of course, recombination is something very, very late.

LEVINS: All we really want in these organisms is that there be some correlation between the state of the system at any two times. It can be a very low correlation—that is, the presence of a given substance only increases very slightly the probability of more of it being plugged—and that means that the system has a very low hereditability, that it would be a very slow evolution, and yet it has the essential properties of what we want.

The question of duplication in the sense of templates can be a relatively recent thing.

FORRO: One point has not been put into this and that is the question of how they actually divide if they do not have something that actually molecularly divides. This is the point where I become entangled on coacervates. The equilibrium picture for coacervates, as I understand it, is to make phases which we can see. These do not tend to divide spontaneously like that—at least the static coacervates do not. I do not know who is working on coacervates that might have accumulating properties of the kind that might be progressive and, therefore, might in a sense have an intermediate metabolism that gives them a driving force, but without that they tend to go to this kind of two-phase business rather than break into droplets.

DANIELLI: On the other hand, I cannot say that at this early stage of the proceedings we really need to subdivide the system. Perhaps this provides two totally different approaches, one where we begin with a subdivided system and one where we begin with a system that is not subdivided.

FORRO: It must subdivide sometime; to evolve something must break apart.

DANIELLI: But things can go a very long time without the need for subdividing.

ENGELBERG: How would selection operate on the system if it does not subdivide?

DANIELLI: Admittedly, I am improvising. Consider a system which catalyzes the polymerization process and another system which catalyzes the hydrolysis, or whatever it may be, of the polymerized product. Then we might have a very large number of variants synthesized to begin with. But if some of these substances which are synthesized have the capacity of being produced by another process or more rapidly than the others, then we tend to end up with a much larger population of the second component than of the less controlled ones, obviously.

Therefore, I think we need to consider the alternative chemical ways in which we could get the sort of particle being postulated, because it is rather improbable that the synthetic processes which prevailed in this early stage are the same as the ones which are used now. I do not see that it is very likely that ATP was available originally for doing all this job; it probably was some other chemical procedure.

McMULLEN: I am not sure about that because we really have been shown that ATP can be formed by irradiation of ADP and inorganic phosphorus, and it has also been shown that at least adenine can be formed under primeval conditions. Therefore, it seems possible to have ATP.

DANIELLI: I do not disagree with that. What I am suggesting is that, under the conditions in which this actually occurred, there may have been something much more efficient than ATP for getting this synthetic process going. And I do not think there is any sense in just assuming there is only one way of doing it, which we would be doing if we looked at an existing biological system.

LEVINS: How far do you think we would get using high-energy arsenate bonds instead, at a higher temperature? And in that case, are we in the only possible world or simply one that is slightly better than the alternatives that were available?

SAGER: Dr. Roberts, what is the relationship between what you call templates (I suppose you mean some form of polynucleotides) and the amino acid-forming protein in the combination?

ROBERTS: There is a relationship between the code symbols and the structure of the amino acids, and it may be a relic from this time.

WATTS-TOBIN: What kind of relationship are you thinking of?

ROBERTS: For example, the ones containing a great deal of μ code for the neutral amino acids. The code sorts out the amino acids according to their structure.

DANIELLI: I will ask Dr. Fox now to introduce us to primitive life.

WATTS-TOBIN: I wonder if I could ask Dr. Roberts a question which I brought up previously. It does not seem to me that if we accept the evidence that the codons for similar amino acids are similar, necessarily implies any chemical similarity between the amino acids themselves and their codons. It obviously has a genetic advantage that mutation is not very easy, and this in itself would tend to drive similar amino acids into having similar codons regardless of any chemical connection.

ROBERTS: I do not think you can prove that at all. It is just suggested. I do not like genetics in it because that would give a chance for the code to diversify during evolution, and it seems to be universal. I would hate to think it is continually modified by evolution.

WATTS-TOBIN: I think if there is one codon for each amino acid, it could not be diversified; but in its initial stage (I am just thinking aloud now), I think the code probably was diversified.

ROBERTS: Originally we probably did not have to bother about tryptophan and histidine.

FOX: Some of you may conclude that I am taking too literally Dr. McMullen's admonition about not being inhibited. Ordinarily, as I found last week for instance, it takes about 105 minutes and much visual aid to develop the subject matter adequately, that I am to try to cover. Dr. Danielli has kindly suggested that we meet the visual problem by circulating some documents (refs. 61 and 62) which I have done. Then, in attempting to present a connected story on the properties that are found in these models of prebiological evolution, I have distributed one sheet entitled "Experiments with a Precellular Model." I had to contain myself sternly this morning because I think the model itself provides a broader way of looking at some of the questions that have been raised and the possible answers to them.

The philosophy of the way to approach the question and the range of questions that may gain some illumination from a precellular model are expressed incompletely in the first paragraph of this abstract. There is, I think, a considerable difference in philosophy with respect to the overall purpose of this conference, that it is primarily analytic in nature whereas the

Abstract

EXPERIMENTS WITH A PRECELLULAR MODEL

Introduction

The bewildering complexity of contemporary cells leads some to invoke the hypothesis of vitalism. An alternate premise is the assumption that complexity evolved from simplicity via natural experiments. Results of model natural experiments are described here.

The thermal polyamino acids, which simply form regular microparticles, result simply themselves from monomers produced nonbiologically. Heretofore, cell models have been produced from biological polymers, such as Oparin's coacervate droplets from gelatin. The data on the thermal polyamino acids challenge many concepts, including the assumption that ordered polymers could not result from heating amino acids, an assumption the speaker once shared. Interpretation of the microspheres described is inseparable from an understanding of their macromolecular matrix.

Properties of Proteinoid Microspheres

Stability (to standing, centrifugation, sectioning).
Microscopic size (1-3μ diam. typically, ——→ 80μ).
Variability in shape (spheres, "buds," filaments).
Uniformity of size (S. D. ± 0.20μ in 2μ particles).
Numerousness (10 mg. yields 10^8 particles).
Stainability (Gram stain, hematoxylin, etc.).
Gram differentiability (+ or - controlled by composition).
Solubility (parallel to G+ and G- bacteria).
Shrinkability (hypertonic solution).
Swellability (hypotonic solution).
Selectivity in retention of outer boundary (optical scope, e.m., time lapse, u.v. time-lapse cinematomicrography).
Selectivity in retention of saccharides (fructose, glucose not retained; glycogen, starch retained; not necessarily permeability phenomena).
Simulation of appearance of cell division (shown by time lapse to be due to processes resembling fission rather than fusion).
Electron micrographability (granular appearance, double layers with increased pH).
ATP-splitting activity (by proper introduction of zinc).

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use of a precellular model is synthetic in both chemical and conceptual senses. Inevitably (and I certainly share some of this feeling and shared it more fully earlier) synthetic models often evoke the scientist's characteristic distrust of inductive reasoning.

I think an evolutionary model has to contend not only with the hypothesis of vitalism, which I find wears many masks and which in my experience appears much more often than some of the mechanists here would think, but also with the presumption of a chaotic disorder in nature, and sometimes, of course, also with a supernaturalistic outlook which in some cases overlaps the vitalistic. Certainly, a supernaturalistic bias is not the explanation for all of the vitalistic outlooks; but any vitalistic outlook, I believe, arises easily because of overwhelming feeling about the bewildering complexity of contemporary cells, and this is what the model attempts to overcome. The experimental model may well be in the same mode, according to the evolutionary premise, as the development of cells themselves in the primitive environment. Both evolve through empiricism.

A recent conference at Wakulla Springs, Florida (ref. 63), covered such subject matter. I think the title I selected for it puts in proper context in another respect the more appropriate way of thinking about this than to talk of it as primitive life or synthetic cells or to use other of what I would call semantogenic words. The title of this conference was "The Origins of Prebiological Systems"; these systems were regarded as model systems by nearly all of the participants, and in the most sophisticated terms of the word "model."

I can see increasingly that I can hardly get into the properties of the microspherical units which arise from this model, and which are listed on the abstract, without saying at least something about the synthetic macromolecules which give rise to them. In fact, I might use as a starting point some of the remarks this morning, particularly the reference to Oparin's coacervate droplets. For those who want to read the rather extensive materials that were provided, much of this is to be found in a preprint, the title of which begins "Electron Micrographs..." (ref. 62); it is a paper invited for a volume commemorating Oparin's 70th birthday.

As pointed out in the introduction of that paper, Oparin, himself, has emphasized quite forthrightly that one of the inadequate aspects of the coacervate droplet as a model is its instability. He has suggested, as has Dr. McMullen, that those relatively instable coacervate droplets had to evolve to more stable forms before cells even appeared as a possibility on the horizon.

The thermal polyamino acids, on the other hand, give rise to units which have a requisite degree of stability from the outset. Further evolution to greater stability need not be visualized. This stability is manifest in the fact that the units obtained can be centrifuged. Figure 20 shows some of these. They are much more uniform than coacervate droplets and they can be centrifuged in the clinical centrifuge; they can retain their integrity indefinitely. They can also be stained, taken up in blocks, and sectioned; and electron micrographs of them can be obtained. The photographs, figures 22-25, are possible because of this stability.

There is also another important difference, which I think is a most crucial one; that is the fact that the material itself, in contrast to the material used by Oparin with the coacervate droplets or by others with coacervate droplets or other cell models, does not come from an organism. Oparin uses gelatin which does come from an organism; but the material that we use is a thermal polyamino acid, and we are able to produce it in the lower range of the molecular weights of proteins and with a content of all of the amino acids that are common to protein. These polymers have many other properties of protein, despite the seemingly violent process of heating the amino acids themselves. This polymerization has to be done under the proper conditions which are dry heat typically at 170° for 6 hours and sufficient glutamic acid and aspartic acid. If we do not have that, we obtain a dark, unworkable mass; but if we do have that, we obtain genuine polyamino acids (ref. 64).

GREEN: When you say dry heat, do you mean no water under these conditions?

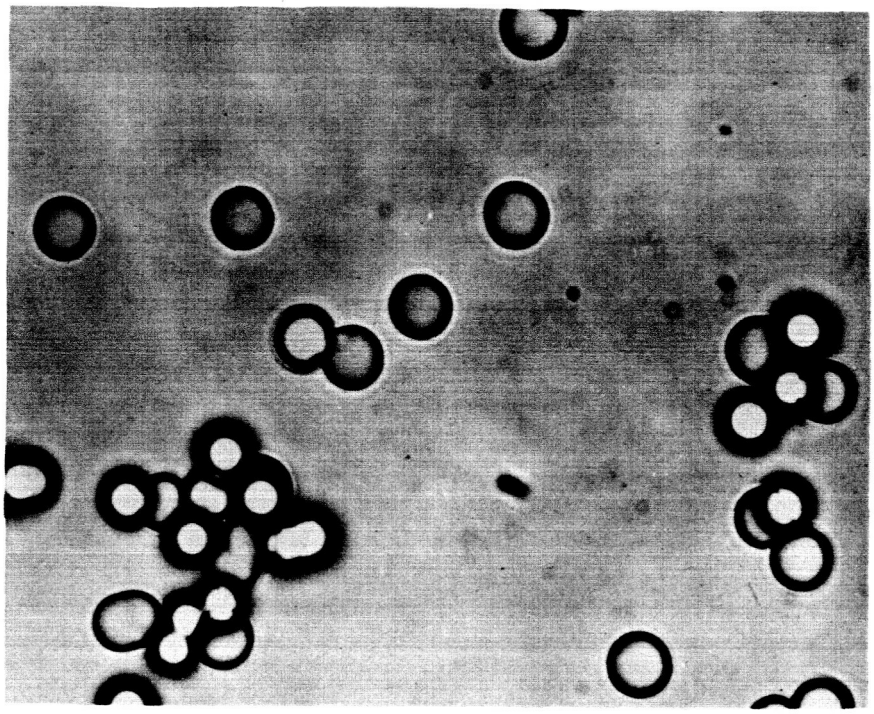


Fig. 20.—Microspheres of thermal proteinoid.

FOX: What I mean is no water initially. There is some byproduct water, of course, but the water that is formed as a byproduct of the peptide bond mostly does not leave the scene of action. There are formed imide linkages that hold onto the water very tenaciously.

ATWOOD: This is a powder, then, when you start?

FOX: Yes. There is another way of doing this, which in some respects is preferable, and that is to use polyphosphoric acid.

POLLARD: Does it help to dry out the solution first?

FOX: No.

POLLARD: If it is just mixed as a powder, is it as good?

FOX: Yes.

McMULLEN: What is the moisture content of the individual amino acid powder?

FOX: This question has been worked out thermodynamically by us (ref. 65) and by Meggy (ref. 66). Thermodynamically, of course, as we add water we force the reaction in the direction of amino acid; in fact, one of the geological aspects of having a temperature like this is that we can hardly have this temperature without having dry conditions.

Another mode is to use polyphosphoric acid or various phosphates; a very interesting one here is calcium phosphate, as well as ATP. Each of these will speed up this copolymerization, and the yields typically run 10 to 40 percent conversion with the highest yields being obtained when phosphates are present.

The minimum temperature necessary can also be lowered by use of polyphosphoric acid to typically 65°.

LEIGH: How long does a proteinoid stay stable under these 170° conditions after it synthesizes? Does it stay around indefinitely or does it change into something else after a while?

FOX: It can remain essentially unchanged through many hours of heating. In the model it need not do so, because rain or tides cool the hot-reaction mixture quickly. Here I am struck by the coefficient of the old Chinese proverb that it would take ten thousand times as long to describe in words what I could show in pictures (ref. 67).

There is something reasonably unique about the phosphate. Many would be surprised that amino acids can be heated for hours at 100° in concentrated sulfuric acid, but it can be done without charring, and the important feature there, as here, is that we must heat dry. The sulfuric acid must be dry, free of water, but with sulfuric acid there is no polymerization. With polyphosphoric acid, or with orthophosphoric acid which is converted by the heating to polyphosphoric acid, there is polymerization and an enhancement of the yield.

The related feature here is that mononucleotides—we found in our own laboratory that cytidylic acid can be polymerized to oligonucleotides with polyphosphoric acid at 65°. Schramm (who has reported on this earlier and also at Wakulla Springs) (ref. 63) has used the ethyl derivative of polyphosphoric acid, also at 65°, and he gets a polynucleotide.

WOESE: Did Schramm work in an aqueous medium or in something like dimethyl formamide?

FOX: No, it is thermal, and it is nonaqueous; it does not use dimethylformamide.

HOFFMAN: How long do you do this with the polyphosphoric acid: It is 6 hours with dry heat. Is it 6 hours with that, too?

FOX: You mean the mononucleotides?

HOFFMAN: No. You said there were two ways.

FOX: It depends on how much yield and how much we want to work up; typically it takes 50 hours for amino acids.

ATWOOD: How long for the polynucleotides, according to Schramm?

FOX: I do not remember what it is for Schramm, but we usually use 2 or 6 hours.

ATWOOD: How long is the polynucleotide chain?

GREEN: How many bases are in one chain?

FOX: I do not recall what he finds. He finds moderately medium molecular weights, nothing as high as would be expected in a natural polynucleotide, but he has fractionated these materials. He has found one fraction which he claimed had been shown by Matthei (ref. 63) to have coding ability. I do not think this is very surprising anymore. Nirenberg reported at the American Chemical Society meeting in September (ref. 68) that polynucleotides synthesized by the more traditional approach by Khorana have coding ability.

GREEN: Recently they have found that a polynucleotide with 10 bases will code.

ATWOOD: I asked this because several people have tried, and have been unable, to obtain polynucleotides by Schramm's method.

FOX: Schramm was aware of this and volunteered the information that the people in this country had not done it under the right conditions.

WOESE: Schramm has shown an electron micrograph of, I believe, polyadenylic acid produced synthetically by his techniques, and it appears to be an extremely long molecule (ref. 69).

FOX: We are satisfied, ourselves, by the work of Schwartz and Bradley in our laboratory that some polymerization can be carried out to ribonuclease-susceptible polymers of mononucleotides by these thermal dry conditions.

POLLARD: Do these proteins show any——

FOX: They are not referred to as proteins. I call them proteinoids; the term "proteinoid" signifies that they are like proteins and at the same time disavows a claim of full identity to protein. Proteinoids as a class have a long list of properties in common with proteins as a class (ref. 74).

POLLARD: Do they act as antigens?

FOX: No, they lack antigenicity, and they also lack helicity; but we have not really looked very hard for either of these properties. All the proteolytic enzymes act on them. They have nutritive quality. We found this first with bacteria; and they have been fed to rats and are being studied in that respect. Krampitz and Knappen have published two papers in Nature on the results of feeding thermal proteinoid to rats (refs. 70 and 71). The thermal polymers have nutritive quality for rats, though much less than casein. They are not toxic in prolonged testing.

YCAS: Do they use l- or dl- amino acids here?

FOX: We can start with l-amino acids and there is some racemization—in fact, quite a lot in some cases. We have found that l-aspartic acid is racemized completely under typical polymerization conditions whereas l-glutamic is racemized about 50 percent under those same conditions. We intend to look at all the other amino acids individually and also to vary the conditions.

SAGER: Are both the d- and l-forms incorporated?

FOX: Yes.

GREEN: I would think that would be mandatory under some of the conditions.

FOX: This effect does not stand in the way of hydrolysis by the proteolytic enzymes or the proteolytic use of them for an organism's nutrition.

WOESE: I should like more information concerning the polyribonucleotides produced under the conditions you have described. It would be interesting to know whether all the backbone linkages are 3'-5', or whether 2'-5' links also occur. One way of getting at this would be to digest the polyribonucleotide with the appropriate ribonuclease and determine whether it is completely degraded to mononucleotides (which it would be were all linkages of the 3'-5' type) or whether a considerable fraction of oligonucleotides are also formed. Do you know whether or not this type of thing has been done?

FOX: What I remember from Schramm's work is that a part of his product is so degraded down to the monomer, but the rest of it is not. This is interpretable as indicating that he is getting a mixture of products, some of which are closer to the natural kind of polymers (ref. 69).

We hope to have our volume out just as promptly as possible, and in that you should find the details you are inquiring about.

BAUTZ: What do you mean by sufficient proportions of aspartic and glutamic acid?

FOX: For workable quantities of polymer, this means at least 4 mols of glutamic acid, 4 mols of aspartic acid per mol of each of the 16 other amino acids. Then as we increase the proportion of aspartic acid, in particular, we increase the yield and the workable quantity. We also increase the proportion of the aspartic acid in the polymer, and in that one criterion the product deviates further from a natural polymer.

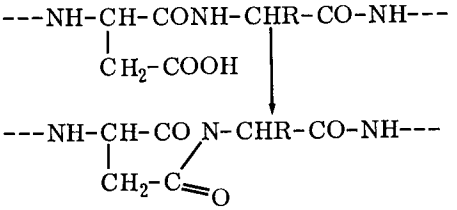
YCAS: These are branched through the carboxyl of glutamic and aspartic?

FOX: There is very little branching in these molecules. Harada and I reported on this feature at the Federation meeting (ref. 72) earlier this year, using a method developed for imide linkages and using Bradbury's (ref. 73) modification of Akabori's hydrazinolysis (ref. 64) method. What we come out with, typically, is a polymer that has a mean molecular weight of, for example, 5000, has one N-terminus and two to four C-termini. This suggests, of course, some branching but not very much.

YCAS: Do you have any idea why it is not branched?

FOX: Yes, I certainly do. The dicarboxylic amino acid that might give rise to branching is aspartic acid, and we can consider this thermal copolyaspartic amino acid. The structure below represents part of a copolymer of aspartic and of another amino acid. Part of the key to getting these results, instead of the hopeless mess otherwise obtained, is to copolymerize a neutral amino acid with aspartic acid, in particular, or aspartic acid and glutamic acid or, as we found more recently, with lysine.

When amino acids are polymerized thermally, most of the aspartic acid residues in the chain react further to give imides. These are easily broken to true peptide bonds by warming with water or by alkaline treatment, but the imide, as you see, permits of no substitution. That explains why there is not more branching. The valency of nitrogen is such that there is just no room for substituents.



This high proportion of imide was observed early in the study by infrared analysis and has been verified more recently by the method for imide determination. I could go through the chemistry of that but I do not think it is germane to this development.

These are some of the basic chemical principles involved in obtaining a very complex polymer in a very simple way. Some of the psychology pertinent to this problem is that the chemist typically thinks of simple processes for simple compounds, complex processes for complex compounds. Here is a simple process, a brutally simple process, for very complex materials that are comparable in complexity to even evolved proteins; in fact, I think they may be more complex than natural proteins.

Since it is a simple process, we can visualize how it could occur in a geological environment. Such syntheses as the carbobenzoxy method are out of the question.

HOFFMAN: Does more branching or cross-linking occur with a different ratio of aspartic to glutamic to one of each of the others than 4:4:1?

FOX: The typical figures of table I show that a 2:2:1-proteinoid and a 2:2:3-proteinoid have a similar degree of branching. The term "2:2:1-" means that in the reaction 2 parts of aspartic acid were used, 2 parts of glutamic acid, and 1 part of the 16 other amino acids in equimolar proportions; these are the basic and neutrals, and we refer to them symbolically as the BN amino acids. Borrowing a term from the polymer chemist, the degree of polymerization for each C terminus in the 2:2:1 runs typically about 12, and for 2:2:3 it also runs about 12. In the analyses that we have done so far, there does not seem to be a very profound influence of the proportion of the aspartic and glutamic acids on the degree of polymerization.

Table I.—Degree of Branching in 2:2:1 and 2:2:3 Proteinoid
[Data from Dr. K. Harada]

Proteinoid	Aspartic acid + glutamic acid All amino acids		Degree of polymerization C-terminus
	Reaction mixture, %	Polymer, %	
2:2:1	80	83	12
2:2:3	57	65	12

GREEN: Is there any restriction with respect to the composition? For example, if there are only four amino acids, would it make much difference in the speed or the extent or is it pretty much independent? In other words, does polymerization occur, regardless?

FOX: We have done many systematic experiments with simpler systems than 18 amino acids simultaneously; and if we have enough aspartic acid and the necessary temperature, polymerization occurs.

GREEN: These are the only requirements?

FOX: These are the most crucial requirements. The result is a little different if there is glutamic acid present as well as aspartic acid. With both dicarboxylic amino acids the reaction is single phase. The pyroglutamic acid formed is a liquid which dissolves the other amino acids. We find that there is a "pecking" order among the amino acids with respect to their tendency to be copolymerized thermally, and it does not matter very much whether we are polymerizing 4 or 18. Alanine and lysine will copolymerize in the largest proportions, typically. There are some variations from one system to another. The addition of polyphosphoric acid makes a difference; changing the temperature makes a difference, but there is for the most part a rather uniform set of relationships.

POLLARD: Is histidine one of the 18?

FOX: Yes. I will come back to that.

FREMONT-SMITH: So we have the origin of the pecking order.

HOFFMAN: Is the same distribution of amino acids found in these proteinoids as in the natural proteins?

FOX: Except for serine and threonine, which are largely decomposed by the thermal treatment. We now know ways to avoid this decomposition in these two amino acids. We do so with hypophosphite which gives a reducing medium; and except for aspartic acid, which tends to come out higher than in proteins in general, the contents fall into the range of individual amino-acid contents.

With aspartic acid we have gotten down to 30 percent or less, and we can get down to lower yields or lower proportions. However, as I indicated before, if we do that we obtain lower yields, and we do not get enough polymer to work with easily.

HOFFMAN: Do the N terminals tend toward the aspartic and glutamic?

FOX: Aspartic acid does not. Glutamic acid shows a decreasing tendency at higher temperatures of polymerization (ref. 74).

DANIELLI: I would like to suggest that questions about the details of experiments should at least be postponed because we are running short of time, and I think Dr. Fox has a type of information that he wants to convey to us.

FOX: Yes, perhaps you can better understand my apprehension about compressing all of this information into 15 minutes.

Dr. Pollard's question about histidine presumably related to the question of catalytic activity, which we do find in these thermal polymers. We find it for an unnatural substrate, p-nitrophenyl acetate. I will not take time for that unless I am able to come back to it, but I would like to answer another question here. We have taken a piece of lava from the Kapoho field on the Island of Hawaii. Instead of using glassware, we placed in a depression in the lava one of our typical mixtures of amino acids and placed that in the oven at 170 degrees for 3 to 4 hours (we have done this a number of times). Then, simulating further one of the kinds of primitive locales that can be visualized geologically for the sequence of events, we have poured upon that a bacteria-free, boiling salt solution or water. In doing so we obtain the typical occurrence that we have in glassware.

On heating we get a suspension of thermal polymer in water, a clear solution to begin with. But as soon as it cools in a matter of minutes, we get vast numbers of these microspherical units which may be regarded as akin to experiments, morphological experiments, in evolution or as models. All of this, through polymerization and spherule formation, occurs in a matter of a few hours. The conditions are simple and geologically widespread.

The related questions are what kinds of properties do we find in such units and what other kinds of properties can we, by attempting to think like a primitive environment and experiment like one, introduce? Of course, the questions asked in this way rest on the original philosophy.

The kinds of properties that are concerned are listed on the abstract. Again, I would emphasize that the uniqueness of this model is that the morphological units are obtained from material that is not itself derived from contemporary cells but, rather, from yet simpler units.

FORRO: Can you take natural peptides of the order of the size synthesized, dry them down and then add your water and stuff and obtain similar units to the ones you describe?

FOX: No. We tried this kind of experiment with a few proteins and have not observed what I would call morphogenic properties of the sort described, but you are the third person to suggest that the molecular weight may be important. That is an experiment we have not done. We have not degraded proteins to a size such as 5000 and carried out such experiments.

If we use as a hypertonic solution calcium salts and magnesium salts at concentrations isomolar with sodium or potassium salt, and at a particular concentration at which there is no effect due to the sodium or potassium salt, shrinkage occurs with calcium or magnesium.

The microspheres are stable to centrifugation, in contrast to coacervate droplets. The microscopic size seen is a few microns (fig. 20). It is possible to make them much larger, up to 80 microns in diameter. Inadvertently, we have seen many shapes, not just the spheres, but some that look like yeast budding.

Some of this is of interest in conjunction with the formed elements that have been reported from meteorites. We have done thousands of experiments of this morphological type and have seen, inadvertently, everything that Claus and Nagy (ref. 75) have reported, except one very strange looking unit.

QUIMBY: The strange looking unit is figure 23 from the original paper on organized elements in carbonaceous chondrites. I refer to the paper from the Fordham group (ref. 75).

FOX: In figure 20 we can see that these are relatively uniform in size, certainly far more so than the coacervate droplets. This feature permits osmotic-type experiments.

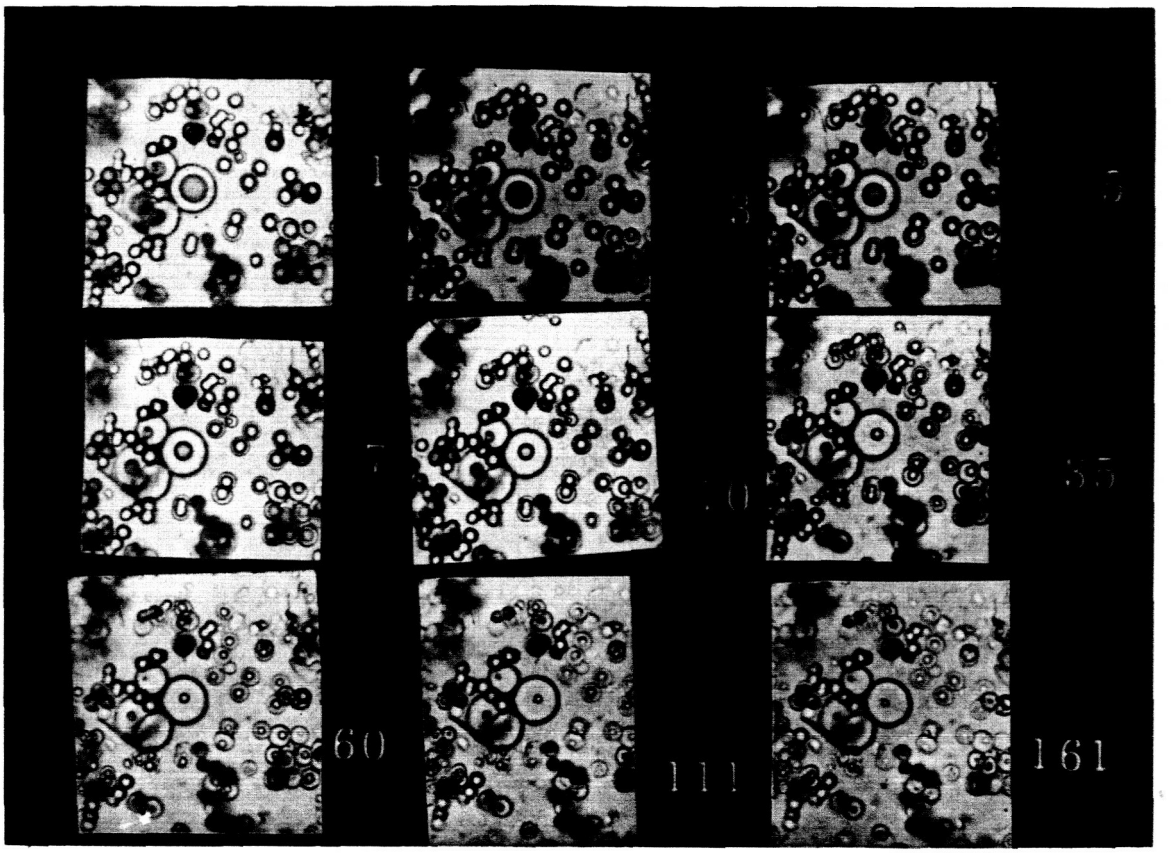


Figure 21.—Time-lapse sequence showing fission in proteinoid microspheres. One is indicated by arrows. The total time sequence is 80 minutes.

I am reluctant to analogize these to osmometers because we must use concentrations of salts to obtain shrinkings and swellings which are about an order of magnitude higher than those which are used for cells, but there seems to be a suggestion of osmotic property here. Here the modification of this property can be visualized, perhaps particularly through the incorporation of lipids.

These are stainable by hematoxylin and the gram stain, and we can also make them in such a way that they stain gram-positive or gram-negative (ref. 76). The essence of this study, which is in accord with one of the many theories of the structural basis for the difference between gram-positive and gram-negative stains, is the proportion of basic amino acid. We find that with a sufficient proportion of polymer rich in basic amino acid the microspheres stain gram-positive, but with less than that they stain gram-negative. Moreover, their solubility characteristics are parallel to those of the gram-negative bacteria soluble in dilute alkali and those of the gram-positive bacteria insoluble in dilute alkali.

We observe some evidence of selectivity in the action of the boundary. One kind of evidence is that we can make these microspheres in the presence of 2-percent fructose, glucose, glycogen, or starch and then wash each of the microspheres four times with water. The ones made in the presence of glucose and fructose retain no carbohydrate. Those made in the presence of starch and glycogen have retained carbohydrate. I would not infer from this that the selectivity is one closely related to permeability. We have not ruled out the possibility, for

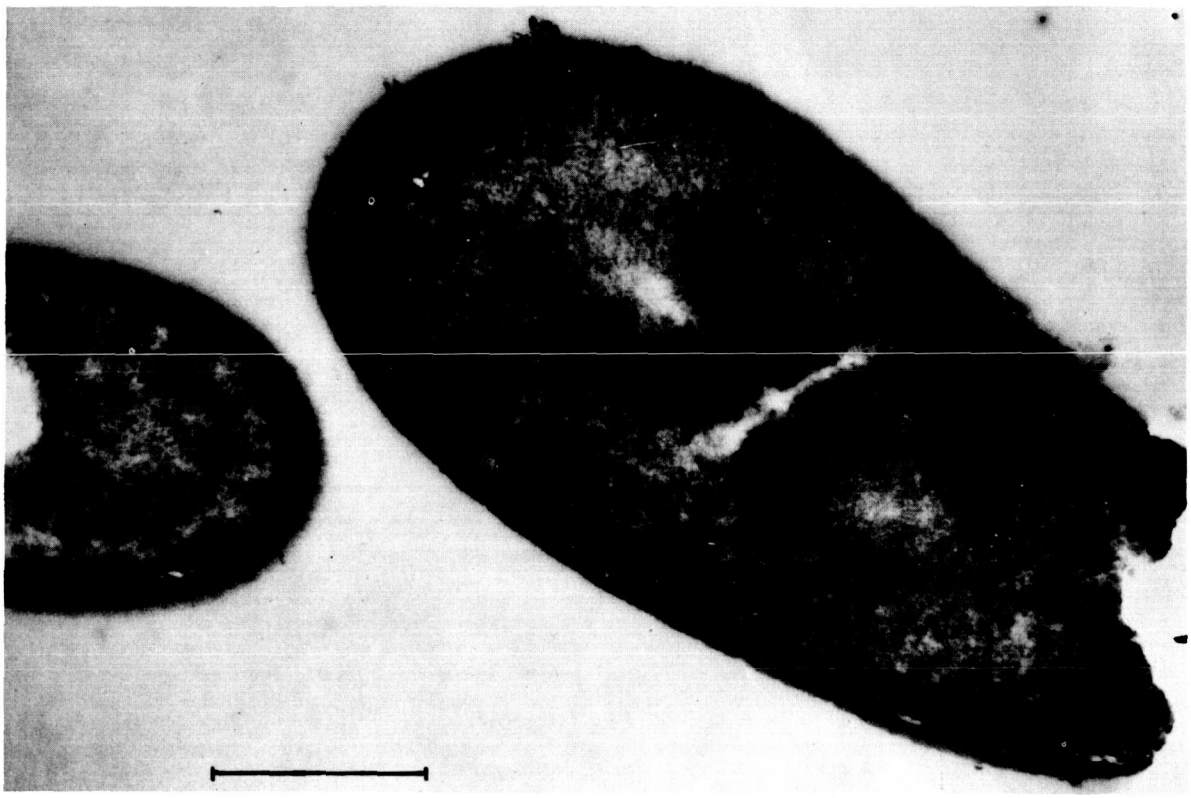


Figure 22.—Electron micrograph of a section of proteinoid microsphere stained with osmium tetroxide and embedded in a block of methacrylate.

instance, that the glycogen and starch are really absorbed on the exterior, but we are experimenting to test whether or not that is so.

However, if we look at the time-lapse sequence of figure 21, a large microsphere can be seen in the interior. This field consists of microspheres that were made in the way I indicated and in which the pH was raised to 5-6. The successive frames in the time-lapse sequence are 30 seconds apart. That is 1 to 2 to 30 seconds. One is 1/2 minute after zero time for taking the pictures; 3 is 1-1/2 minutes, et cetera.

We can see that the center is disappearing and yet the outer boundary remains; this, of course, is a kind of selective action and a first suggestion, at least, of one of the properties that would be associated with membranes.

Also, we can see some evidence here for Brownian motion because the residual center in this figure 21, is off from the geometric center in different directions in successive pictures.

This kind of result is also found in the electron micrograph of figure 22. The electron micrographs of figures 23-25 show some microspheres made from proteinoid in which the pH has been raised by adding McIlwain's buffer, pH 5 to 6, under the cover glass, to a suspension of the usual microspheres. Figure 23 shows a sequence; all of these, of course, caught in methacrylate blocks and stained with osmic acid, show schematically a sequence. The one at the bottom is one in which this deterioration of the center has not proceeded very far; the one in the upper left-hand corner would represent a second stage; the one in the lower left-hand corner a third stage; and then either of the other two a fourth stage. Yet, as can be noticed the boundary remains. This, then, is an electron micrographic verification of the optical

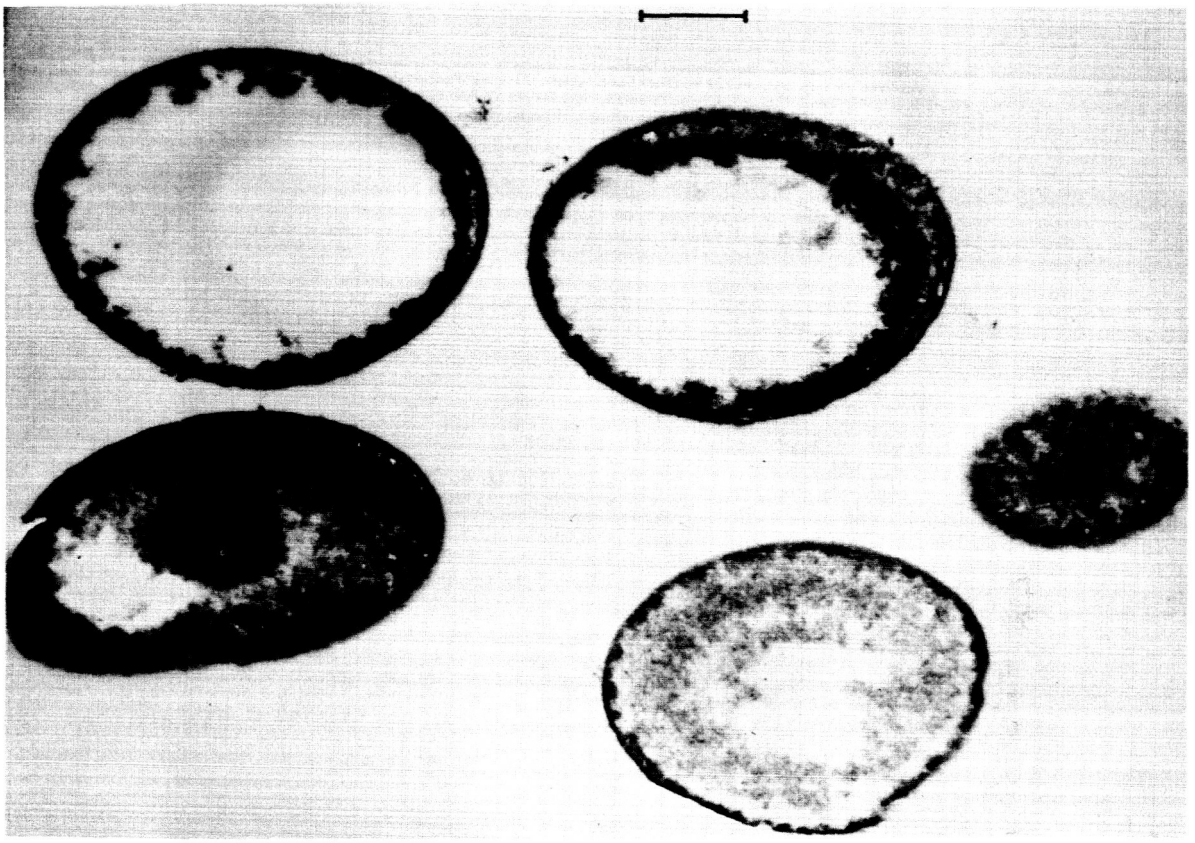


Figure 23.—Five proteinoid microspheres prepared as for figure 22.

micrographic kind of result, since the optical micrograph, of course has certain inadequacies with regard to a conclusion like this. Incidentally, on each of these the marker is 1 micron. These are all by T. Fukushima. These sections have been made with an ultratome and they are typically about 800 angstroms thick. Also, Dr. Philip Montgomery at the University of Texas has followed this experiment through with ultraviolet cinematography and has observed the same sort of phenomenon. *

Since we have here the pictures in the paper for the Oparin commemorative volume, we might look more at those. Figures 24 and 25 show a kind of double layer and there is either no, or essentially no, lipid present. At first, we were concerned with the absence of phospholipid, although I think Dr. Green's experiments help to make this possibility a little clearer; but we also thought in terms of the fact that there might very well be the necessary hydrocarbon character in the side chains of the neutral amino acids, of which there is a goodly proportion in the material. We have, indeed, done a number of analyses on the whole microsphere and compared these with analyses of the shell after diffusion of the interior and washing, and we do not find any difference in amino acid profile as yet.

ATWOOD: Can you be certain that this double-wall structure is not a fixation artifact?

FOX: I do not think we can be any more certain of this than we can be with the true cell.

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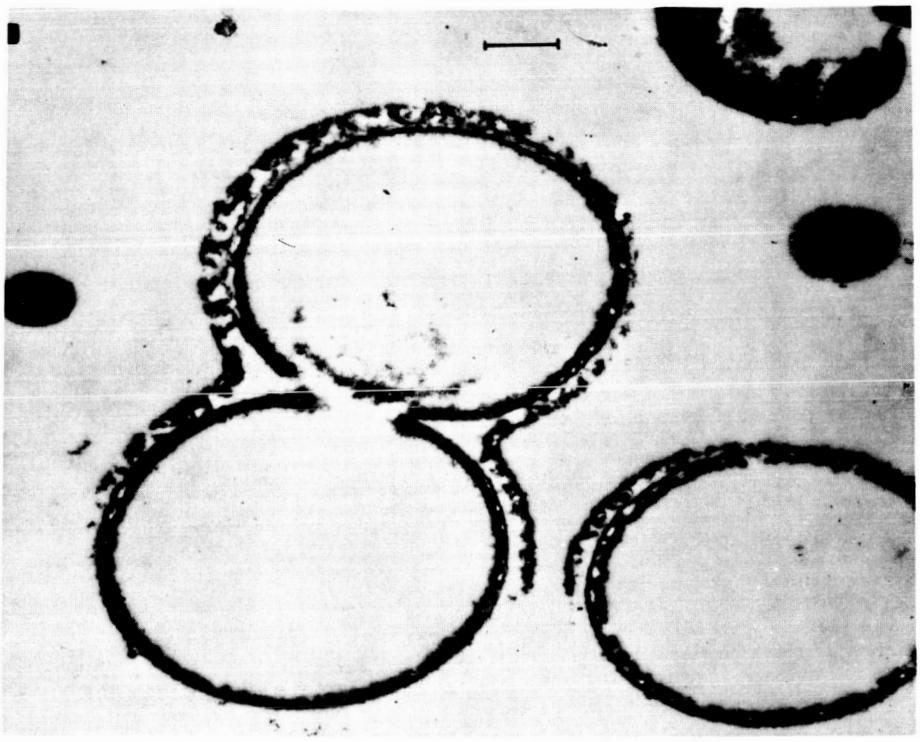
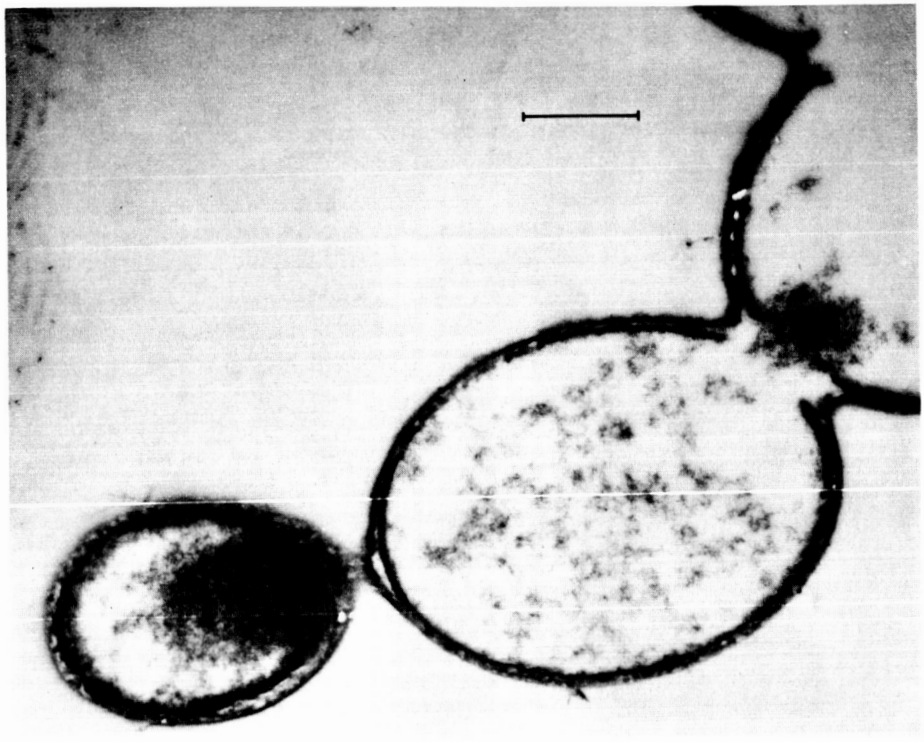
*Montgomery, P. O'B., personal communication, 1963.





Figures 24 and 25.—Two photomicrographs of proteinoid microspheres subjected to raised pH and carried through electron micrography. Double layers are evident.

ATWOOD: I asked the question facetiously.

GRENNELL: Do you mean that electron microscopy always makes the same mistake?

FOX: Yes, I think we must have that reservation in both instances, the synthetic or the evolved material.

I will just refer to the last property which we found most recently and which we feel is of more interest than any of the others, singly speaking; that is that we have been able to introduce ATP-splitting activity into these microspheres. We worked for about a year and a half on this, and we know many ways in which we cannot do it reproducibly; but it can be managed reproducibly by adding freshly prepared zinc hydroxide gel to the proteinoid being heated in the aqueous solution. Both the zinc hydroxide and the proteinoid go into solution and microspheres separate out that are different from the others we have seen (and we have seen many) but that are of themselves morphologically very interesting and which clearly, after careful washing with water, have retained the ability to split ATP.

BAUTZ: I do not want to sound mean when I ask how high the control is because it is known that metals do split phosphates.

FOX: Yes, that is right. In fact, that is the basis on which we did the experiment. I am not prepared to say, and I would not want you to draw the inference, that there is more activity on a specific zinc weight basis in these units than would be obtained in an ordinary inorganic salt of zinc. On the other hand, I cannot say the opposite, and there are experiments under way that are aimed at answering this question definitively.

I think the significant aspect in a prebiological context is that it is possible to incorporate a metal that has ATP-splitting activity in a unit that has separated from its environment.

DANIELLI: Thank you, Dr. Fox. Dr. Pollard is now going to attempt the heroic task of summarizing what should be deduced from the day and a half's activity.

POLLARD: I think, Mr. Chairman, there is going to be ample opportunity for you to hear my voice today. In trying to summarize this conference thus far, there are several things I must do concerning the part dealing with the cell. One is to try to connect some thoughts regarding de novo cell synthesis and theoretical biology, and another is to synthesize some thoughts regarding theoretical biology.

First, I would be unwise to dwell very long on the reaction to the problem of de novo cell synthesis. All present have heard the evidence, the discussions, have seen what kind of situation we are confronted with, and thus have come to your own conclusions. I will simply give my conclusions, not that these represent a summary or that they have any authority at all.

My conclusions can be summarized in the following manner: I think that, coming from quite different areas, my own account and that of Dr. Forro, Dr. Morowitz, Dr. Green, and Dr. Slonimski all indicate that the present-day cell is a highly organized, specially constructed machine that has been selected by evolution. It works; it almost inevitably appears that it works in an accurate cyclical way. About 5 years from now, we probably can look to maps for E. coli on which not only will the synthesis of the DNA be perfectly, definitely arranged in terms of the cell cycle, but also each of the enzymes as they are produced in this cell will be known as to the moment when they arise. We will know when the cell membrane is made as it goes along; and we will know when the cell wall is made as it goes along. All of these things will be part of a very regular, smooth, nice pattern of a living cell.

Frankly, the thought of making a cell de novo overwhelms me. I will say this, too, that if we can make such a cell de novo if we only have to find, as Dr. Roberts says, the three enzymes, the three polymerases, and the other ingredients and put them together, and then this marvelous process results—then I will go one stage further. I believe that indeed we will

find biology has produced one more law of nature. There will be a principle of organization not yet apparent in the physical world which will have taken over and made possible this very remarkable process.

Thus, I feel it is well worthwhile to play with systems like this. I congratulate Dr. Fox on actually having done something about it. I must admit that in terms of systems, which I think many of us have thought were partly symbol-producing pictures (which both surprised and interested me), I do not know that I feel this is the key to the formation of the present-day cell, and Dr. Fox has certainly never said so because he speaks of prebiological evolution throughout his story.

I think it is interesting, and I would also urge Dr. Morowitz to deviate from his 15 slaves, or whatever they are that operate in the PPLO factory, or at least to deviate 2 of them to that very nice suggestion of squirting the insides of the PPLO into a drop of oil and watching what happens. In this way he could perhaps convince Dr. Grenell that this was, indeed, not contamination, which is what he said. I think this is probably worthwhile.

The other thing I think I realize is that the best approach to the synthesis of a cell de novo is to imagine something in between the very primitive situation which would simply follow what Dr. Fox has been suggesting and the present-day situation. It seems that a place where theory may very well play an important role is in telling us what partial success to look for. In other words, it is possible that we will not at first choose the right epoch in the evolution of the cell to look for the place to synthesize, but perhaps in choosing the wrong epoch we will get some part of the way. Thus, the question is, What will we predict as being a success in that context and will we be guided to look for something later or something earlier? Of course, there is also the question as to whether we can find the right kind of cell to synthesize right now. This is one of the things that I think Dr. Morowitz has stressed.

Other than to summarize as I have, I can say nothing because I think the problem is clearly presented to us. I think there is a great deal to work on, and I think to say anything more would be presumptuous.

I can turn to the other aspect of this conference, that of theoretical problems. The first thing that occurred to me during the conference was something that bothers us all the time. For instance, I noticed some conflict between Dr. Woese and Dr. Green. Dr. Woese suggested if we took a very thin mica layer (and by the way they are indeed very thin when we get them thin), damaged or altered it in some way, and then used it as a membrane, we could obtain remarkable concentrations of ions. Of course we are clearly not looking at a contractile membrane nor at what most of us feel we mean when we operationally speak of a permease. Dr. Green felt this was irrelevant, and I think perhaps I do, too. But it is interesting that one of the things that should be done as part of the service, which theoretical biologists can so to speak give to the biological community, is to examine and characterize as many purely physical processes as possible. And they should also write their conclusions clearly so that these things will actually be available. This, by the way, is not notably done by theorists, which is a sad thing.

For example, concerning this mica membrane, what probably incurred a moment of resentment on Dr. Green's part was this—and certainly I would join in that, and here I am speaking for myself—fundamentally, what we need to say is: Does your mica system have properties which could conceivably come close to the ones observed in biology? Does it have anything like them? And doubtless it does. The point is, does it have enough of them that we should stop even thinking about the contractile process, that we should stop thinking about this rather odd stoichiometry he observed, and, so to speak, force ourselves to discard this hypothesis first?

We could work on material, authoritative enough relationships, enough descriptions. There are many processes—membrane separations, foldings of protein, foldings of DNA,

mechanisms by which things can move in the cell by purely physical methods, mechanisms by which two mitochondria can collide and join as two soap bubbles can collide and join—and all of these purely physical mechanisms should be examined. If we look at the first, the 1933 Cold Spring Harbor symposium on surface phenomena, we notice the remarkable fact that the early days of theoretical biology were 30 years ago, because at that symposium there was a great deal of this kind of discussion, which was much more respectable in those days than it is now. I suggest that part of the task of theoretical biology would be to restore the respectability, perhaps not in terms of interpretation of biology, but in terms of a necessary chore to clear out these available thinkings and systems so that the possible miracles of biology can become clearer.

I have divided the remaining into a series of topics, each of which I think contains a certain amount of relationship. The first one concerns essentially the basic DNA story. The theory of DNA rotation, the actual facts and the possibility of DNA rotation, the energy required for it, and so on, should be worked on by someone again. There have been a few suggestions about it, a few mechanisms of ways of twisting and so forth, but I believe it needs, not necessarily more scholarly work, but a larger accounting. I think the topology of DNA replication is a subject for theoretical study. We know that the DNA has both to uncoil and to untwist, and we know that it has to take place in a bacterial cell inside a cylinder. In the case of Dr. Morowitz's PPLO, it has to take place in a very, very tiny region, in the center, presumably, of this very small object of something like 1/10-micron diameter; this seems to place strong topological restrictions on what it is doing. Someone with a sense of form and of the relationship between forms would do well to examine possible ways in which this can be done, because certainly something as small as the PPLO case, perhaps similar to Dr. Green's small particles, has only one way in which it can be assembled.

I think that the description here of the work by Dr. Seymour Benzer, in which the mutation rate for phage is found to be extremely high in certain cases and much less high in others, may very well have within a genetic map a physical relationship to the actual physical state of the DNA, which is suggested and which, like many theoretical problems, has no originality in its origin but needs to be worked up. Perhaps there is already available a very clean theoretical problem having good data that could be compared with the theory.

The question concerning what happens when the messenger is made on the DNA is, I think, important. In our discussions we evolved the idea that only momentarily was there separation; this, I think, suggested a process which certainly could be studied in terms of molecular scale study and thus would definitely need theory. The problem of the elasticity of DNA, which may not be easy to measure experimentally, could be approached theoretically. Enough facts are known about DNA that the elasticity of DNA could actually be calculated theoretically. I also think that the precision of synthesis is important. For instance, this mechanism of separation of the DNA, the momentary separation, suggests that perhaps the precision of the messenger's synthesis may not be as great as the precision of the synthesis of DNA itself. This should be examined theoretically and something formulated about it.

Taking another group of problems, not in the DNA group, the problem of the transport of protein to organelle regions arises. At lunch today (and here I am putting on record our discussion as Dr. McMullen suggested) Dr. Green told me that he is able to examine essentially liters of mitochondria; in these liters of mitochondria he was not likely to say there was no DNA and no RNA, but I think he would like to say there is no significant DNA and no significant RNA. On the other hand, we do know that mitochondria grow or become more (I will put it that way, a little differently perhaps from growing; they become more), and they become more out of quite actively characterized protein. In fact, Dr. Slonimski is quite clear on the fact there are genetic mechanisms by which the protein can be characterized in these mitochondria, and genetic mechanisms by which the mitochondria are made must almost automatically

not be in the mitochondria itself. If there is no nucleic acid, it must not be within the mitochondrion. Because all of this material (which is organized in a most significant way into those quadruple doughnuts we saw making the layers) somehow has to get there, I think the transport of the protein into the system and what happens to it is interesting. After considering that, the assembly of the mitochondrion becomes a majestic problem of theoretical biology and one that I suggest is alone sufficient to occupy the time of many theorists.

Concerning the system of energy transfer in the enzyme blocks, Dr. Woese suggested that perhaps we could have a specific operation that made possible the organization of one molecule with respect to another over some distance. This is something which needs to be thought about, and thus the process of multiple enzyme systems, which I have characterized as enzyme blocks, should be considered.

Unquestionably, we have channels in which metabolites are moving. The cristae in mitochondria represent one such channel. My strange sort of growing of the membrane of a bacterial cell, which is an imagined structure, and the surface structures of both the outside and the inside of mitochondria as described by Dr. Green require, almost certainly, restrictive regions within which metabolites are transferred. The way this transfer takes place, I think, is theory.

Leading to another topic is the interesting question of the packing of protein in lipid. Obviously, this is a key to all of the membrane structures that we have heard of. While we have very informative pictures about protein and lipid structures, I think that this area, again, is theoretically discussable. For instance, I gather from Dr. Green that it is the hydrophobic groups of protein that are found within the lipid. He also told me that the actual activity of the enzymatic processes probably takes place not so much in protein but in the lipid. This is something definitely worth theoretical consideration.

I was fascinated by the translocase, the idea of this being in some way related to something in bond under actomyosin, these things that Dr. Szent-Gyorgyi suggested are sitting on the membrane. This is a fine idea, and again I believe it needs to be thought about. As soon as these three ions are put on the surface, a very potent and interesting electrical structure develops. As we speak of contraction, I cannot help asking, What are they contracting against? As I began to think of the actual twisting process that would occur and of the looping sort of law and the rest of the membrane, I began to have a strange visualization of this twisting process on the surface. And I must admit I would like to see someone work this out and reassure me.

If we consider the other problems, I think Dr. Slonimski produced a beautiful one. He pointed out that in vitro the complementarity, which genetically has great power in this study, is available. I am not quite sure he can do all those things in 2 weeks. If so, I think perhaps we should import a few lab technicians. At any rate, there is no question that a theoretical hypothesis is made possible; moreover, should we not, as theorists, know how to put together potent parts of proteins in solution in a fairly definite way? What is the matter with us? Why are we throwing up our hands on a problem like this? It seems to me a delightful question for theory to consider.

In connection with, but along a somewhat different line, I think a general attempt should be made to look at the theory of protein secondary and tertiary structure. I do not think that the number of proteins we now know, which is 8 or 10 in great detail, should really be so complicated and so difficult that the mechanisms of all the inevitability of folding and of general folding behavior of protein should not be something we could theorize about. I would say this is a perfectly good thing to talk about, too.

Last (and I do not know whether we can work this up for this conference), as I have sat listening, what impresses me, as it has for all the years I have tried to work in biology, is the difficulty of finding a knowledge. In this room we are not really well selected for variety

and precision of knowledge. We are selected for an interest in theoretical biology, which is not necessarily the same thing at all, but obviously by merely going around the room we can satisfy ourselves on many points—for instance, nucleic acid in the mitochondrion. I would like in this conference, or through a grant by NASA and AEC, to support a deliberate attempt to make approximately a 50-page compendium of what might be an extension of Dr. Morowitz's five points. It could begin with the invariants as he spoke of them, move out from the invariants into what might be called the firm knowledge, then move out into the reasonably firm knowledge, and then into the very peripheral thing. If this were compiled in a 50-page book that could be perused and considered, I think it would be very good.

This idea is not quite so amusing because the AIBS and several other agencies have put these glossaries of nucleic acid terms and protein terms together. If you do not by any chance have these in your library, get them. They cost $1 and the graduate students have "borrowed" them so systematically that we have to keep ordering a new supply. I think that the purpose of this array of suitable knowledge would be to pick more than just those two subjects and put them all together.

DANIELLI: I did promise Dr. Pittendrigh a minute.

PITTENDRIGH: I would like to respond in part to my own question last night of what theoretical biology is because I implied by asking the question that I thought it was worthwhile. The few comments I am going to make will be quite deliberately provocative; and since I have only a few minutes, I am going to read some notes I have written in the last few minutes. Otherwise, I might talk until 5 o'clock.

I want to be so bold as to start from what might seem trivially obvious, that the task of theoretical biology is to explain the organization of the kind found in living systems and that this explanation, like all other cases, will be achieved only by theory. The question is whether there is anything peculiar to living systems that makes theoretical biology in any way different from theoretical physics. This might immediately suggest that I am about to flirt with vitalism—and I certainly am not.

First, if we note the biologist's basic axiom that all living systems are evolved complexities of matter (whatever that is), then vitalism is excluded. Second, physical theory is the major arsenal of basic explanatory tools to which the biologist can turn. That is all clear; however, there is more. The organization we wish to explain is the product of a long historical process, and it is a process we know much about, not so much as to the phyletic details but as to the principles involved. The nature of the historical process is to fix the first sufficient novelty that will enhance reproductive competence, and that fixation may turn out in historical perspective to be a commitment that catalyzed all later changes in the organization. That is to say, the process of selection chooses one from an ensemble often containing many physically sufficient solutions to the problem.

Purely historical considerations (in the sense I am using here) have played so dominant a role in the determination of organization at higher (multicellular) levels that it is wholly unreasonable to believe it has played a minor role in the much longer history of the cell's organization. For example, is the function performed by proporphyrin in so many specific molecules, and so many specific different functions, such that only the proporphyrin can do it, or is it merely that the function to be fulfilled can be fulfilled in each case by a molecule for which the cell already has a synthetic apparatus on hand and thus uses it? Again, with respect to adenine in so many different cofactors, in ATP itself, and in the information storage and translation devices, is there a physical aspect on each process which is uniquely fulfilled by the physical properties of adenine? Or again, has the cell, given the apparatus to synthesize adenine, used it simply because it is sufficient, not necessary?

What I am saying is that a major task of the theoretical biologist is to disentangle those features of his organization (the cell) that are not only physically sufficient but physically necessary to discharge the function demanded from those that are physically sufficient but not necessary and present there for purely historical vis-a-vis physical reasons. This distinction is necessary even if our goal is, for instance, a purely physical explanation of living organization.

There is an alternative to the "purely physical" explanation that is not yet vitalistic. That explanation involves the purely historical considerations derived from the probabilistic rather than the deterministic nature of the historical process that has led to living systems. This distinction is also crucial to any rational approach to the search for extraterrestrial life. Thus, to the extent that we can demonstrate that the major concrete features of the living systems we know are physically necessary and not only sufficient, we can to that extent use with greater confidence easily conceived life detection systems that are basically chemical detectors. To the extent that historical accident has early selected a catalyzing single alternative from the many possible, we are confronted with the more difficult task of designing function detectors.

More generally, I believe that theoretical biology should be concerned first with identifying the functional prerequisites of the minimum living organization and then with pursuing the formidable task of asking whether or not the way cells we know fulfill these basic functions is a way that is not only physically sufficient but physically necessary. (Please forgive the repetitions for these are notes.) By functional prerequisites I mean such things, for instance, as information storage and accumulation of novelty, information replication, information transmission (these are all different), and information translation systems. As a functional prerequisite we also need controlled energy transfer systems. There are other more general prerequisites in an organized system, such as the need to preclude spontaneous processes except on specific permission. Incidentally, we need principles of organization if we are to make progress.

In summary, I have been trying to say that we need not only physical theory in the narrow sense but we also need selection theory in a very broad sense if we are to hope for a complete explanation of a system which has arisen by a process of selection. Unless we understand that, we are not going to explain it. Thus, for instance, even if we could synthesize a cell de novo we would not have answered the question as to whether other systems elsewhere could exist with a different organization but equally alive.

PART II. ASPECTS OF THEORETICAL ECOLOGY

CHAIRMAN: R. LEVINS

LEVINS: Going now to population biology, we are dealing with an area where three separate disciplines are coming together: evolution, population genetics, and population ecology. These fields came about through different lines of study and generally made different assumptions. The population geneticist generally considers the heterogeneous population with its different genes, usually in a uniform environment, without considering population density or age composition even though population number does become important and without being too concerned with other species except indirectly as they affect survival value. Population ecology, on the other hand, is more interested in heterogenous environments, in systems of many species, and in populations where we must make distinctions as to ages of individuals. But population ecology generally assumes that each population is genetically homogeneous. And, finally, the evolutionary study emphasizes the temporal and historical changes taking place.

We would like to combine these; however, then we have a model requiring heterogeneous populations, many of them, in environments that are changing in time and in space. Such a model produces a number of analytical difficulties. We end up with mathematical models that are virtually unworkable. We have random processes, simultaneous random processes, that are nonlinear. The environment appears here as a random variable. It is all right to treat a fluctuating random environment in a simple genetic situation and consider the fluctuations in gene frequency. However, for a population to derive any benefit from responding to natural selection, from changing, some correlation must exist between the environments of successive generations. If we allow a correlation between the environments of successive generations and the change in general frequency is no longer a Markov process, it becomes a stochastic process generated by a Markov process. We have virtually no theory for dealing with such situations; thus, in this area we must appeal to the mathematicians to devise systematically a particular class of non-Markov processes that we need.

A second difficulty in the mathematical problem of manipulation is that the parameters appearing in these equations are virtually unmeasurable. We have a large number of species interacting and a coefficient of interaction between each pair, but in most instances we have no hope of accurately estimating any of these terms. It would be impossible for a group of population ecologists to have a discussion similar to ours earlier where speakers would ask how many angstroms is that or how big is the molecule, but then we are dealing with equations, the parameters of which we do not know and cannot measure.

Finally, suppose we do know the parameters and we do solve the equations; we would get something that is virtually uninterpretable. Solving a set of simultaneous equations, quotients of determinants, each of which is a sum of products of these unknown parameters, does not provide us with any nice intuitive meaning. Therefore, this field of population biology is taking a somewhat different strategic approach. We are not trying to set up equations to measure all the parameters and solve them; we are looking for something else, and there are several possibilities. First of all, we are interested not so much in a solution to the equation as in the character of the equation, such as whether there is stable equilibrium, or more than one stable equilibrium, or steady oscillations.

Some here may be familiar with the arguments used in considering equilibrium in competition between two species. If two axes on a graph represent the abundance of species 1 and 2, the quarter plane can be divided into two regions. When the points lie in one region, species X1 declines; when the point lies within the other region, species X1 increases. A second line divides the plane in the same way for the other species, and it is the rate of the intersection

which determines whether we have stable equilibrium. If Y increases more rapidly along the X-axis where X is very abundant and if X increases more rapidly along the Y-axis, we can have a stable equilibrium. The interesting thing about such a model is that it is robust in the sense that it is very resistant to changes. We assume straight lines. The straight lines assume that the coefficients of interaction between these cells are constant. The logistic equation has been criticized, but if we make reasonable curves it does not change at all as long as the curves cross each other in the same way and the same relation exists at the intercepts of the axis. This is nice because that means that we can express our results in conditions of inequality. It is equivalent to saying that each species inhibits the growth of its own members whether or not it inhibits the growth of members of the other species, and inequality of this kind is much easier to reassure ourselves about than exact equality.

SAGER: Is that a general example?

ENGELBERG: Is that a general statement you are making that each species inhibits its own members?

LEVINS: No, it does not always occur. I am saying that is a condition for equilibrium between the two species.

FRIEDENBERG: When referring to species interaction, do you assume that there is competition between species at all times?

LEVINS: Yes, there is first the species in the same environment competing for a food, or only partially, or each one contaminating its environment but more for its own members than for others. I am not saying this is an experiment we can necessarily make, but it is the inference that comes from here.

A second kind of problem that we deal with compares whole groups of species and tries to make generalizations about them. I use as an example some investigations that we have carried out on the adaptive significance of migration. In the old literature migration between populations was considered harmful because of the swamping effect, which prevents a population from adapting to its local environment. This is true provided the environments of the two populations are different and constant. When we consider the opposite situation—the two populations of the same species, living in different places, within environments that are on the average the same but each one fluctuating in time—in that case the migration of genes between populations has the effect of damping the response of each local population to fluctuations in the environment. The damping of this response is advantageous if the fluctuations are ephemeral or of short duration. Only when there are long-term changes in the environment is response to selection advantageous.

It turns out that environment changes of long duration are also widespread geographically. This means that the fluctuations in the environment which will last a long time will tend to occur simultaneously in the two populations in question. When that happens, the interchange of genes between them does not dampen the response at all. From this we can conclude that the adaptive significance of migration is to permit the two populations each to respond to the long-term widespread fluctuations of the environment while damping their response to the short-term local ephemeral changes. If this is true, then, we can say that temporal fluctuations in the environment increase the advantage of gene flow, but spatial gradient between the environments decreases the advantage of gene flow. From this we can then conclude that the optimum degree of gene flow is greater for those species having a high temporal variance in their environment compared to the spatial gradients.

This hypothesis can be tested by considering the whole flora in a region such as the transection from the coast up to the high mountains of Puerto Rico. All the plants have the same gradient, average temperatures, and average rainfall; but the environment is much more

variable for a weed, for an annual, or for a plant of the open country than it is for trees of the forest climax. The reason is that the long duration of a forest damps out the average fluctuations of environment. The forest environment itself retains water and is therefore relatively stable to drought conditions. Because the seeds of forest trees are large, the next generation will most likely be rooted in the same soil type as its parent. From such considerations we can predict the amount of gene flow between populations for weeds and for trees. We get inequalities which lead us to expect differences between whole large categories of the flora or fauna. Similar generalizations could be made concerning life span and possibly individual size and other properties of this sort.

Another way of circumventing the analytic difficulties of this complex ecological system is to concern ourselves with properties of a system itself rather than properties of the individual species. This could be done in the following way: let the rate of change of each species be given by $r_o x_i(K-x_i-\Sigma_j x_j\alpha_{ij})/K$ where x_i is the abundance of the i-th species, K the carrying capacity of the environment for that species, and α_{ij} the effects of species j on species i (which may depend on the environment). Thus we have a matrix of the α_{ij} terms (with ones along the principal diagonal) which describe the structure of the community. The many properties of the community as a whole depend on the properties of this matrix. The stability of this community is related to the eigenvalues of the matrix. The trouble is that we do not know the eigenvalues because we have said nothing yet about the α's. We can, however, make several kinds of statements. The average eigenvalue for this community is 1 because of the 1's along the principal diagonal. The community will be unstable if there is any eigenvalue which is negative. The greater the variance of the eigenvalues, the more likely is a negative value; and we can set an upper limit on the variance of the eigenvalues compatible with stability.

It could be shown that the variance of the eigenvalue of this community is equal to $\overline{\alpha}_{ij}^2$ plus the covariance of $\alpha_{ij}\,\alpha_{ji}$. This enables us to analyze the stability in terms of a kind of ecological relation, the reason being that when we consider a pair of competitors the α is a positive and the α^2 is positive and in general we would expect a positive covariance between them.

The more species i affects j, the more likely it is that j also affects i. With a pair of competitors on the other hand, the alpha of one is positive and the alpha of the other is negative. It will be a negative covariance between them. This reduces the total variance and, in fact, the total variance may become negative. When the variance of the eigenvalue is negative, this means there are imaginary terms and this, in turn, is indicative of cyclical conditions in the community.

Finally, we can consider the evolution of this system. We begin by assuming that species are invading a new area, an instance such as Krakatoa, and that there is no correlation among the α's except for the symmetric pairs α_{ij} and α_{ji}. On the other hand, the effect of each α on the survival of the species is proportional to its cofactor of the variance. This means that the intensity of selection on the α is proportional to its cofactor, which in turn means that covariance will appear between α's and cofactors as the community matures.

We think this will give us some indication of the maturity of a community in subsequent evolution; thus we are able to pass from the demographic equilibrium obtained from solving these equations to the evolutionary equilibrium. We can show that in a competitive community the optimum structure, which is also the direction of evolution, is one in which the covariant terms vanish. This comes about when all the α's become equal. That in turn can be interpreted as meaning it is advantageous for a species to spread the amount of competition over many different species instead of only a few and that the stable communities, the mature communities, will have a species competing with one other species for food, a different one for nesting site, another one for some other activity. Thus, by working purely on the level of the community, we are able to make a series of generalizations which lead to inequality predictions between mature and immature communities, between communities that have a complex

trophic structure and communities that consist primarily of competing species on the same trophic level.

The fundamental hypothesis that has been guiding this research is that whereas specific structures of organisms and specific physiological processes represent adaptations to specific qualities of the environment, either physical or biotic, the structures of populations, the amount of polymorphism, the mutation rate, and the amount of migration are adaptations to the statistical structure of the environment in space and time. The optimum condition of a population in terms of polymorphism and these other traits will depend on the variance of the environment in any one locality, the covariance between environments of successive types, the average gradient from place to place, and characteristics of this sort; therefore, it is possible to develop an integrated theory of adaptive systems.

All of this work, then, is taking place on the level of the population as a whole, of the community as a whole, and for our purposes the gene enters into our considerations only as a frequency or as an average adaptive value. In population genetics it does not matter what the genes are made of, and nothing that molecular biology can discover at the present time will have any bearing at all on the level of the population itself. However, there are a few points of contact between the different levels, and these represent a somewhat different orientation, a somewhat different approach, to problems of population ecology. All the complex chemical and physiological properties within the individual come up to the population level as an average viability effect. That does not mean we are indifferent to the biological underpinnings of these systems. In fact, since we are concerned with evolution, the question arises, What can we reasonably expect in terms of the mutation rate? This depends on the relationship between the physical nature of the genetic information and the physiological and developmental processes that are controlled by it. There are other areas of relationship between these different levels as well. One point of view, a completely different approach from the one I have taken so far, is held by Dr. Wesley, who is considering thermodynamic properties of the community and studying energy relations. He believes apparently many of the dynamics of the population can be determined, not in terms of the biological coefficients that we have identified but in terms of the same physical concepts that can be used in nonliving systems.

In the work that Dr. Kerner is going to describe, there is a slightly different approach. He is also working with a physical model except that, whereas Dr. Wesley is using the same temperature or energy in physical and biological systems, I think Dr. Kerner is using analogous systems. The temperature that appears in his description of population dynamics is not the same that we have in a physical system. Rather, it is an analog playing the same role in the equations which refer to fluctuations in the abundance of species. Therefore, the gap between the different levels is being bridged by isomorphic equations, equations having the same form and in which there is a 1:1 correspondence between the terms on one level and those on another.

I think perhaps this is also close to what Dr. Garfinkel will discuss, where the system of equations as described by chemical relations between the cell are going to be applied also to systems on an ecological level. I am not sure exactly in what way.

At this point, before I ask them to present their arguments, I think Mr. Leigh should mention some things about the relationship between the levels.

ODUM: Could I interrupt to clarify a point? Were you saying, essentially, that as units are added to make things more complex—as more species are added to systems, for example—great disorder is produced and, therefore, you find it impossible to analyze?

LEVINS: I did not say anything about disorder. I just said it gives us problems.

ODUM: That means disorder, does it not? If the problems cannot be solved, that means there is great disorder. We all know that living systems, when they become more complex, actually go in the other direction, toward becoming more orderly thermodynamically.

LEVINS: My statement was not about living systems but about us—that there are problems we cannot solve.

ODUM: You were also talking about populations that you felt could not possibly be analyzed mathematically because of the continuing addition of species. Later you seemed to refute this idea by saying we only have to first lump these new functions into a new term and then we can analyze it. I wanted to get it clear that homeostasis does exist, does it not, at all levels of organization. As we go from one level to another, processes become more, not less orderly.

LEVINS: I think that order in the system and ease of studying the system are two very different things, and structure does not mean stability. We can have ecological communities which are quite unstable; yet, we can extract a great deal of information from them.

ODUM: My point is that regulation of a different type exists at different levels, and this regulation produces order of a type which makes it applicable, then, to study or to put it into mathematical form.

WESLEY: It should be easier to solve the problem, the more the species.

ODUM: The population is not necessarily more difficult to study than the cell. I would say it might be easier. This is a false notion that adding units to the living system makes it more difficult. This does not follow, does it?

LEVINS: It becomes more difficult if we use the same tools as before.

ODUM: Yes, but we must use different tools and different conceptual components at different levels of study.

Another way to study a population, which may be better, is to start at the other end of the spectrum. In other words, start with the ecosystem in which the population is functioning. If we are going to study mitochondria, we start with the place in which it lives, the cell, and then we come down to the mitochondria. Later we try to build one up from the macromolecules. Thus we can also approach populations by starting with the system in which it lives. This provides an approach which may be easier to handle than trying to start with the isolated individual, because populations are not assembled that way. They are assembled as part of a system just as the mitochondria are assembled as part of the cell.

LEVINS: The fact is that historically biology has always started building on all floors at once.

ODUM: I believe we must look at each level as approachable directly, not necessarily through long stair steps from other levels.

LEVINS: In fact, that is a statement of the problem and not the solution. The questions we want to study are the laws that are operating on each level, and we are also interested in seeing how the levels look together.

ODUM: That is right, but the horizontal rather than the vertical locking together of levels is a better way to look at it.

LEIGH: I just wanted to make a few remarks about some of the interests and problems the molecular biologists and the ecologist-evolutionary theorists might have in common. The first such common interest has perhaps already been looked at. The evolutionary theorist would be very interested in some sort of an understanding of the hot spot problem, some sort of understanding of how the mutation rate can be altered at a given nucleotide by altering the nucleotide arrangement in its neighborhood. I am thinking in terms of the possibility of rearranging nucleotides around a given point consistent with the degeneracy of the code in order to obtain another gene putting out the same enzyme as the original one, but having a different mutation

rate. The control of mutation rates is a matter of very great interest to the evolutionary theorist: here we have a case where a result obtained in one field can be directly applied to another (ref. 77).

The second question is one of similar method, perhaps. Ecologists have long been interested in constructing energy-flow diagrams of a community, seeing what proportion of the energy flows through a given component of the community, and so forth. I wonder if the construction of such an energy-flow diagram for the cell might not be useful. For one thing, we might argue that the energy-flow diagram of the cell would resemble closely the information-flow diagram. Thus, if we could, for example, determine that most of the energy in the cell goes into basic syntheses of amino acids, nucleotides, proteins, et cetera, and that none or very little of it is directed into ordering these proteins into the characteristic form and structure of the cell, then we might justifiably conclude that the steps requiring external regulation, presumably in the form of direct transmission of information from the DNA, are those steps leading to the synthesis of macromolecules and that natural selection has worked sufficiently on the design of the macromolecules relevant to the structure of the cell so that the processes arranging these molecules in their proper order are now autonomous ones taking place without explicit interference from the DNA (this may not have been true, however, earlier in the history of life).

The last question I had in mind was one of common language, the existence of problems at various levels which, prima facie, would seem dissimilar, but which in reality would require a common language for solution. In arginine synthesis, if I recall correctly, there is a feedback inhibition which has a characteristic overshoot, so that the curve of concentration would follow a damped oscillation with a period somewhat like 6 hours. If we have a number of feedback processes running simultaneously which may interact, we may sometimes obtain from the interaction of all these oscillations a resultant rhythm which is more accurate than any of its components would be in isolation. We thereby obtain a chemical clock, which may serve to order in time the various processes which take place in the cell. These possibilities have been adumbrated by those working on the clock problem.

The formalism required to attack this problem would be similar to that required to attack certain problems in neurophysiology where the alpha rhythm of the brain, which has a certain characteristic frequency, seems to serve as a gating mechanism to synchronize the effect on a neuron of impulses reaching it at nearly the same time. Again, the function of the rhythm would seem to be the organization of processes in time, and again, the rhythm has been ascribed to the interaction of a number of damped oscillations.

It has been pointed out by Pringle (ref. 78) that, from a purely formal point of view, these problems bear a remarkable similarity to problems involving evolution in a species consisting of a number of partially isolated subpopulations which interact with each other to some degree but which are not entirely linked with each other.

Here we would have areas in three or four separate branches of biology seeming to require a common language which, once put into decent mathematical form, might apply to other problems. The idea here is that one of our problems in theoretical biology is to create a common body of mathematical method, a body of mathematical method which might perhaps be peculiar to the study of organized systems. Before at least a nucleus of such a body of mathematics is developed, we in theoretical biology will be suffering under a great disadvantage.

At this time I would like to add some references in addition to Kimura (ref. 77) and Pringle (ref. 78) which I believe to be of general significance. This list includes some of the most beautiful papers I have ever read: they form a natural unit, a collective point of view toward theoretical biology, and I have often wished they could be published together as such in an inexpensive edition.

For the mathematical theory of evolution in general, consult the following:

Fisher, R. A.: The Genetical Theory of Natural Selection. Clarendon Press (Oxford), 1930.
Wright, S.: Evolution in Mendelian Population. Vol. XVI of Genetics, 1931, pp. 97-159.

Some idea of the uses of energy flow diagrams in ecology, and also of the uses of the community approach in ecology in general, is given in the following:

Hutchinson, G. E.: Homage to Santa Rosalia, or Why There Are So Many Kinds of Animals. Vol. XCIII of American Naturalist, 1959, pp. 145-159.
MacArthur, R. H.: Fluctuations in Animal Populations and a Measure of Community Stability. Vol. XXXV of Ecology, 1955, pp. 533-536.

For the possible significance of formalisms involving systems of loosely coupled nonlinear oscillators, see the following:

Wiener, N.: Cybernetics. John Wiley & Sons and M. I. T. 1961.
Pittendrigh, C. S.: On Temporal Organization in Living Systems. Harvey Lectures, Series 56, 1961, pp. 93-125.

ATWOOD: Some other similarities between the interests of molecular biologists and evolutionists can be mentioned. One concerns the rate of fixation of new mutations and the stability of the base composition. In bacteria, the fixation of a new mutation is known as a population changeover. It can be seen in populations maintained in the chemostat, for example, when the frequency of some selectively neutral mutant is followed. Instead of increasing continuously by mutation pressure, the frequency of such a mutant will periodically drop to a lower level, from which it resumes its approach to mutational equilibrium. These shifts are the indicators of population changeovers. At each changeover a selected mutation is fixed, and the population passes through the very narrow bottleneck of one or a few cells in which the selected mutation occurred. This is an occult bottleneck because the total population size does not change significantly during the process. It results in extinction of the various selectively neutral mutants that have accumulated in the past, and these begin to accumulate again in the new selected population. This process, which Ryan has called orthoselection, will continue indefinitely unless a condition is reached where none of the possible mutations is selected; such a condition seems rather unlikely. I should add that a theory of orthoselection for metazoans has not yet been worked out.

LEVINS: You get this effect in parthenogenetic clones.

ATWOOD: Yes, and also to some extent in sexual organisms, since regions closely linked to a selected mutation will go through a bottleneck when that mutation is fixed.

More recently there has been some interest in the stability of base composition in bacteria. I will try to show how orthoselection is relevant to this problem. In bacteria, base compositions of the DNA can be very different in different genera, in contrast to the situation in metazoans where they tend to be more or less alike within a phylum. We can speculate on the time that would be required for the base composition to diverge from that of a common ancestor to the present state of variability among bacteria. First, it may be noted that the base composition can change appreciably without a proportional change in the amino acid composition. This is made possible by the degeneracy of the code, whereby one amino acid is represented by more than one triplet of bases (codon). It has been found that the overall amino acid composition among bacteria does change significantly with their base composition, but not as much as it would if the code were nondegenerate. Thus, the base composition can, in principle, be shifted toward high or low A-T content by base-substitution mutations that do not change the amino acid composition. If the rates of mutation in the directions A-T $\rightleftharpoons$, G-C

are determined by the internal milieu, they may progress toward different equilibria in different organisms. The rates of approach toward such equilibria with substitution rates within the range of specific locus mutation rates have been calculated. The base composition is an extremely stable character. However, under a continuous regime of orthoselection the base composition may be more or less stable than expected, depending on the size of the genome and the average selective differential between successive populations. The theory remains to be formulated. The rate of changeover, as represented for example by the rate of peaking of an unselected mutant in the chemostat, would give a minimal value for the rate of fixation of mutations.

QUIMBY: I calculate those peaks to be once in every 300 minutes. Is that correct?

ATWOOD: No, no! Of the order of 1/100 generations. The generation time may be from 30 minutes up.

QUIMBY: I am sorry, I was thinking of phage.

ATWOOD: Perhaps the growth rate has a theoretical limit.

CROW: Let me ask a question. The mutants being discussed are those that themselves change the growth rate, or are they the mutants that happen to be in the same cell which has a change in growth rate for some other reason?

ATWOOD: The minimum number fixed comprises the selected mutations, but coincident mutations will also be fixed. Under constant conditions, the growth rate per se cannot remain the selected feature indefinitely. I surmise that interactions between successive populations would be the most important basis for orthoselection in the long run.

BAUTZ: This depends also on the mutation rate, which we do not know. In early times there may have been a rapid rate, a much higher rate of mutation.

ATWOOD: Yes. Mistake mutations may have been more frequent when the polymerases were not so highly evolved.

Another question (unrelated to the foregoing) arises from a comparison of the number of cell lethals in higher organisms with the number of genes necessary for the minimal cell that Dr. Morowitz outlined. In some organisms experiments are possible that distinguish between lethal mutations and cell-lethal mutations. A zygote that is homozygous for a lethal mutation will stop developing at some stage, but with a cell lethal the lethal phenotype is expressed at the cell level; that is, cell lethals affect the maintenance of the cell itself, whereas zygote lethals affect differentiation. Evidence suggests that the number of cell lethals is larger than the number of genes required for the hypothetical minimal cell, whereas at first approximation it might be expected to be the same. Is that point clear?

MOROWITZ: No.

ATWOOD: The requirements for a viable but nondifferentiating cell should be the polymerases, translation system, structural macromolecules, and a fraction of the intermediary metabolism that the environment cannot provide—in other words, requirements similar to those for the minimal cell.

LEVINS: Once we have a nonminimal cell, is it not true that things become lethal?

ATWOOD: New things become lethal.

LEVINS: And yet any gene that would suppress the head in the mammal would certainly be lethal.

ATWOOD: A mutation precluding that form of differentiation would not necessarily preclude cell viability and the capacity for indefinite replication.

PITTENDRIGH: There may be N steps necessary to keep the system going; but if we evolved the system sufficiently, there may be N plus X steps of killing the system.

ATWOOD: Yes. It seems obvious that the total number of lethals will increase as the complexity of the organism increases, but an increase in cell lethals is not entirely obvious a priori; there is room here for theoretical speculation.

GARFINKEL: I am going to discuss the simulation of ecological systems and the comparison of simulated ecological systems with simulated biochemical systems. In doing the ecological part of this work, I have had the advice and encouragement of Dr. Robert MacArthur, the mathematical ecologist at the University of Pennsylvania who was invited here but could not come.

This discussion logically breaks down into three parts: What do we have to work with? What can we do with it? And what results have we obtained? I believe that several of my reprints were distributed to the participants. The first of these (ref. 79) describes the computer routine that we were using for this work, which effectively is something that enables the computer to translate automatically chemical equations into differential equations and then solve them. Almost all the user has to worry about is the organization of the system and the numbers to put into it; the computer will proceed from there. This is not a completely accurate description, but it is good enough.

There are other methods available beyond the one described in that paper, because not everything can be described in terms of classical chemical kinetics on the assumption that everything is free to diffuse. We encounter biochemical systems that are not free to diffuse. (In fact, Dr. Green has described the most important of them this morning.) In order to deal with these we have prepared a somewhat different type of program that allows us to represent individual molecules within the memory of the computer and to specify how they interact both with each other and with things that are free to move around (ref. 80).

In this type of system we can set up an array of status numbers and specify that the molecule represented by one, which may be a hemoglobin molecule, can react with another one which in turn can react with another one. We can also specify that this one react with the molecule next to that one and, if we want, also with the molecule next to that one. We can also specify different rates. In this any complex set of interactions can be carried out.

Very recently ("recently" means the first useful output was obtained this week) another program was prepared to do this type of work (it traces its ancestry to the programs used to simulate steel mills) by Margaret Dayhoff, whom I think Dr. Klein is also interested in working with. This program allows a wide variety of events to be put in, but it is not specialized enough to do enzyme kinetics or related things (ref. 81).

I should say a word about the economics of this problem. Margaret Dayhoff and I have both made estimates of the expense of simulating a PPLO cell with a computer in this way. She came to the conclusion that we could about do it in real time; that is, have the computer going as fast as the cell does, with a 7090. Being more experienced and more cynical, I think it would take the largest and fastest computer now commercially available, the 6600. Thus, in effect, we would need approximately $3,000,000 to $6,000,000 worth of hardware, which would pretty well fill this room, to simulate the behavior of a cell that is just barely visible under the microscope. But this is not as bad as it sounds because the cost in computer time for doing the average useful experiment is something like $10 considering that it would be a complicated experiment and more would be spent in preparing for it and interpreting it than in doing the experiment itself, I would think this is reasonable.

We have these kinds of programs. Now what can we do with them? A computer is a beast that is very obedient and very ignorant. It does exactly what it is told to do. If I may give a few extreme examples, we could start writing an equation composed of the Gettysburg Address,

as one of my programers once did when he needed a convenient series of symbolic names, and we could then proceed to put numbers into it obtained from the telephone book, as Dr. Klein and I once did when we were studying the effects of the numerical values on the stability of the system, and the computer will usually give some kind of an answer.

If a way can be found to represent an ecological system in terms of differential equations that can be written this way (and we can write most differential equations this way), that ecological system can be simulated and something done with it. And this is exactly what we have done.

The work we did started from the theoretical work of Lotka (ref. 82) and Volterra (ref. 83) who effectively assumed that the rate at which two species A and B interact is given by a differential equation of the type

$$\frac{dA}{dt} = - K(A)(B)$$

where (A) and (B) are the populations of A and B. This is the same differential equation that describes chemical interactions.

Some people object to the use of this, particularly Ken Watt* who has just moved to the University of California at Davis, and who undoubtedly will be doing ecological simulation there. His objection is (in part) that this does not take proper account of the effect of population size on growth rate. In particular Volterra (ref. 83) assumed that the rate at which species A reproduces is proportional to the population of A. Watt feels this is true only in special cases, and our experiments are in agreement with him; that is, it was quite difficult to build anything approaching a stable ecological model that did not have some kind of regulation on the growth of the various species.

It also turned out that this regulation was usually as close to the species in question as we could possibly make it. In biochemists' terms, we had things at the level of product inhibitions or at the next step after that while in many biochemical systems the feedback is over a longer distance, so that we may very well have a large set of enzymes making a final product which then inhibits the first enzyme. This we have not been able to simulate in ecological systems. Although we have not really worked sufficiently with complex enough systems to say it is impossible, I do think it is improbable, and I can even offer a biochemical example to show why.

The glycolytic pathway has the sort of structure where many different things are coming in at the beginning and many different things are going back out for quite a while. Then there is a long stretch further down where there are no branch points. The enzymes at the beginning of the system are very highly regulated; in fact, phosphofructokinase, which is the third enzyme in the system, has so many active enzyme inhibitors that it is effectively taking orders from all but two of the other enzymes in the pathway. But there is a group of five enzymes near the end which act almost as if they were a unit. It has been shown by Pette et al. (ref. 84) that in all tissues having active glycolytic systems these last few enzymes occur in constant ratio to each other, and there are almost no inhibitors known for any of them. There may be three inhibitors for the four or five enzymes in this portion of the pathway.

This is a straight pathway with no branches. We do not often find that in an ecological system. It is very rare that a given insect, for example, is eaten by only one other insect and eats only one other insect. To be effective, then, the feedback loops would have to be shorter than they need be in biochemical systems. This was the first kind of finding we got, and this is a contrast between ecological and biochemical systems.

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*K. E. F. Watt, personal communication.

In the course of experimenting with ecological systems, we found that they do have one property in common with biochemical systems; namely, they are not excessively sensitive to the exact numbers of the rates that are put in. If we change the rate constant in a biochemical system slightly, it will act almost as it did before. If we do this in an ecological system, the same is true with the one exception where there are two competing species and one is driven to extinction; changing a rate constant slightly may change the identify of the loser.

We also tried in a moderately complex system (the most complex we could squeeze into the computer we were working with) to see whether the number of interactions had any great effect on the stability and behavior of the system. Again this was not carried as far as we would like, but we did find that we could throw out a fair number of weak interactions, the sort of thing where one species eats another which is not its primary food, without changing the system very much. On the other hand, on eliminating certain of these weak interactions, we moved to instability, which means either wildly growing fluctuations or something going to extinction.

In a biochemical system I should think that if we could eliminate a few of these things with moderate damage, perhaps eliminating more would cause more harmful effects. I recall that Dr. Klein and the late Henry Quastler were doing work of this type with an analog computer at Brookhaven.* When we started out, we invariably obtained results where things did not become extinct in a hurry but instead went through cycles, with the cycles getting wider with time. This suggested that our ecological models had clock properties, and I am sure it turns out that they do. So do the real systems, and some are very well known. In particular, our attention was focused on the rabbit-lynx cycle in Canada (ref. 86) which has a cycle length of about 9 or 10 years. This is not very different from another naturally occurring cycle, the so-called sun-spot cycle which gives weather disturbances over a period of 11 and some odd years.

In other words, there are two distinct cycles of slightly differing lengths. The question immediately occurred to us, Are these clock properties strong enough to resist being dragged along with a stronger cycle of a slightly different wave length? We were able to try this out by effectively varying certain of the rate constants sinusoidally. If we have a reproduction equation of the type grass yields more grass, we put in a seasonal factor (which we just called SES); and since the amplitude of that sine curve was not very strong, this system behaved much as it did before. As we made the amplitude stronger, species began to decrease in number or even in the extreme case to go extinct, but we were unable to change the cycle length of the ecological system very much. Thus, even a system as simple as that does have a clock property. This is a system involving only six species: three plants, two herbivores and one carnivore—or more accurately, one omnivore because we could not make a pure carnivore (ref. 86).

PITTENDRIGH: By what great piece of luck did your choice of parameters give you the 8 years? Did you adjust these to get these precise answers?

GARFINKEL: Oh, no. I am not saying that this is a real system which matches the rabbit-lynx system. I am just saying that it has a periodicity, and a different periodicity will not coerce the first one. There has been much discussion on biological clocks within individual organisms, but it is very rare to find this at the biochemical level.

I should mention also that in building this system we assumed that the animals can exist in two nutritional states which are conveniently described as fed and starved and that a fed animal will become a starved one if it does not get to eat and a starved one will die of starvation if it does not get to eat. We have built several models without this assumption (i.e., with

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*H. Morowitz and H. Quastler, personal communication.

only one nutritional state); thus far, grossly, they have looked like the models with the assumption, but they have in general been more stable.

We did obtain one finding which I am afraid will be of interest to NASA, probably in a rather painful way. We have been discussing thus far the question of whether a small cell is less complicated than a large one, the presently indicated answer being that a large cell contains more repetitions of the same kind of unit. We tried this kind of experiment with our ecological system. In order to do that, we had to define the total mass of protoplasm in the system. If we do not say anything about it, we are presumably looking at a small piece of an indefinitely large system. What we did was to assign a value for the amount of protoplasm of each species and write our equations in such a way that the amount of protoplasm remained constant.

If the size of the ecological system was large, we found that the system behaved the same way as it did when the size was infinite. But as we decreased the size of the system by decreasing the amount of protoplasm, the species started to be squeezed out from the highest trophic level on down; that is, the carnivore became extinct first and the herbivores started heading toward extinction also as we decreased the protoplasm further.

This is in accordance with what can be observed of real ecological systems. If we look at an isolated small system, a small island, it may have only plants in it and not much in the way of herbivores; whereas larger systems have herbivores, still larger ones have first-order carnivores, and a continent is needed to support a second-order predator.

Unfortunately, we may have to face the situation of a spacecraft as an isolated ecosystem, and in these terms it is a very small one. The chance of having an ecosystem in a spacecraft large enough to contain an appreciable number of men as first-order predators, which is probably what we usually average out to be, is not very good for the near future. It would be a gigantic task, and I hope we can find some way for astronauts to avoid having to eat algae whenever out in space. It would be rough on them.

KERNER: I was a little confused on one point when you were comparing biochemical kinetic systems and ecological systems. Do the biochemical systems being studied have the property of being chemically kinetically closed and therefore reaching a final equilibrium point, or were they open and therefore oscillatory indefinitely?

GARFINKEL: No, they were closed. We have rarely taken them to equilibrium. It has usually been to a steady state, but there is another point that I forgot to mention. As I said, the ecological systems have clock properties, which are difficult to eliminate. The biochemical systems do not have clock properties, although it is known that some biochemical systems must have them. I suspect that by juggling with the rate constants we could get the glycolytic system to show clock behavior, at least for a short time.

PITTENDRIGH: I wonder if you would go back to this point. You made the interesting statement that the frequency of one of the systems you described was very insensitive to change. Change of what? I missed this.

LEVINS: The frequency of his predation from the outside.

PITTENDRIGH: What was the frequency of the act of perturbation from the outside relative to the spontaneous frequency?

GARFINKEL: About twice as long.

PITTENDRIGH: What about perturbations that come closer to the natural frequency systems?

GARFINKEL: This we did not follow up. At the time the experiment cost us about $50 each. We can dream them up with the greatest of ease, but to carry them out is a strain on the budget. I hope we will be able to carry this further.

ODUM: I think we are doing what we did this morning. We are again discussing research that is interesting to individuals. To my way of thinking, in the theoretical approach we should ask not how ecological levels are the same as molecular levels; we have already covered a nice list of those things. Now the question is, Are there any ways or any principles or theoretical considerations in which ecological levels are different? You have mentioned one—clock properties present at this level and not at the molecular level.

GARFINKEL: I mentioned two. The other is the effect of size on complexity.

ODUM: We should make lists of these properties that are different or additional. Our purpose, as I see it, is to find out what is new or what is different at ecological levels. Otherwise, we feed back and talk about DNA some more. We would only be saying that nucleci acids are the same at both cell and ecosystem levels, which we all know. But the question is, What is now unique or different about populations, communities and ecosystems? What else can we now list besides the clock properties which have to be studied at ecological levels and cannot be studied at the molecular level? In other words, we can say the proper study of ecological levels is ecological levels, and not cells.

GARFINKEL: I can suggest a third one—the effect of a lag in reproduction on stability. It has been shown by some Australian workers with pencil and paper methods (ref. 87) that a delay of offspring coming in an ecological system will make it unstable, and apparently the longer the delay the greater the instability. When we simulated this in the simplest possible way—namely, that A yields A immature and then A immature grows up to A again—we found that the slower this process is, the more unstable the system.

We then tried the reverse experiment. Suppose that A immature, even though it takes its time about becoming A, affects the properties of the system by virtue of its being there (this is reasonably true of mammals, for example, where the parents feed the young); we found that this could indeed exercise a stabilizing effect.

I do not know of any comparable biochemical situation.

LEVINS: We will now adjourn for dinner and reconvene at 8:00 p.m. In parting, I will just suggest that the time lag in a biochemical system may be sufficient to delay diffusion.

GARFINKEL: It can but I have never heard of it causing instability by so doing. As a matter of fact, another paper (ref. 88) of the two that I distributed is on exactly this subject. It shows what happens when a couple of reactants are diffusing through a heterogeneous field of mitochondria and the resulting changes that this causes, but I would not call that instability. It is only a second-order difference.

I would like to say a few words more on what can be done in population ecology with simulation techniques. We can simulate a system that is much too complex to be handled analytically. In particular, we can handle things like different genotypes in the same population because we can perfectly well compare two subspecies, A and A* for example, which differ at some gene and as a result have different properties; we can even mate the two and obtain hybrids and follow their behavior.

If desired, we can study migration between one region and another by just calling the animals in the two regions different names. We can vary the environment, and we can vary it randomly because, as I said, these rate constants are not really constant; if we want to put a random variation on them, this is not at all difficult. I think that a few more of the problems Dr. Levins has proposed can be dealt with in this way. I am not saying this is easy to do. Inevitably, a person trying to do experiments of this sort will make mistakes in particulars

and it will be expensive, but it converts things from a near impossibility into something which is just difficult and perhaps nasty, and that applies to so much of what we do.*

(The conference recessed at 5:20 p.m.)

The conference reconvened at 8:10 p.m. with Dr. James F. Danielli, Chairman, presiding.

LEVINS: Before Dr. Kerner's presentation, I would like to focus your attention especially on the alternative models for approaching the same kind of system, which are an array of different species linked together. Note that Dr. Kerner, in speaking as a physicist, is making more precise assumptions concerning some of the parameters and obtaining more precise results numerically, at the expense of departing somewhat from the natural situation. Therefore, we have here a whole spectrum of precision of error coming in from the model, as opposed to error coming in from the lack of regularity as in the presentation I gave before.

KERNER: I would like to begin by saying a word about models. I think that the object of greatest importance in all theoretical physics might be an object that does not exist at all in nature. This is the humble harmonic oscillator. Although no harmonic oscillator exists in nature, in the early part of this century Lorentz** made an atom—a very crude one, in fact just an oscillator—and it was able to predict quite properly and in a basically correct way from the empirical point of view what was known to be true of optical dispersion for a gas of atoms. In fact, the harmonic oscillator was the instrument by which Planck essentially invented the basis of quantum mechanics, and it still stands as a principal tool by which we can uncover some of the essential mathematical points of all of quantum theory and of all of field theory. Yet, as I said, the harmonic oscillator does not exist in nature.

Thus I would like to consider another kind of oscillator, one which has already been mentioned. I would just like to attempt to draw a few parallels between what may be done with this type of oscillator and what is done with oscillators in physics. I will be quite frank and say at the outset that this is probably not a good idea. What is needed is some thinking de novo and this is thinking "de oldo," unfortunately; nonetheless, it may be at least entertaining to see what might be done.

Also, there is one tool of methodology which has proven itself very useful in physics, and I would like to show by an example that this same tool may be of interest in biological problems. In this connection, I should also like to mention what might be considered a distinct prejudice in favor of theorizing from the top down instead of from the bottom up.

In a certain sense ecology is at the top of the biological complexities; yet, remarkably enough, theoretically it is perhaps the oldest part of biology, and some of the essential aspects of the theory of ecology date back to Benjamin Franklin and probably much earlier. We can also ask about theorizing from the top, that we try to theorize from the top down as far as may be done while at the same time trying to theorize from the bottom up. The difficulty with theorizing from the bottom up is that it is very difficult, as I am sure we are all aware; moreover, it may not be feasible on principle to carry it very far.

Therefore, let me begin with the biological oscillator, the one that has been mentioned already, the Volterra oscillator, and I will try to avoid writing any mathematics because that

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*Most of the work here described was carried out during the tenure of a Research Career Development Award from the National Institutes of Health, and variously supported by the National Institutes of Health and the Office of Naval Research.

**All references noted by Dr. Kerner appear in Annuals N. Y. Acad. Sci., vol. 96, 1962, pp. 975-984.

is not the point. Let us just consider a particular predator-prey situation, the classic one which is understood in some respects experimentally being paramecium in culture in a solution of sugar which also contains yeast—a very famous microcosm that was studied by Gause in 1930. What one finds is this: The predator, of course, will start eating the prey and will soon overeat; then the prey will be so few in number that the predator will not have enough to eat and so will begin to decline. The prey now has a chance to increase again; thus we have a cyclical process.

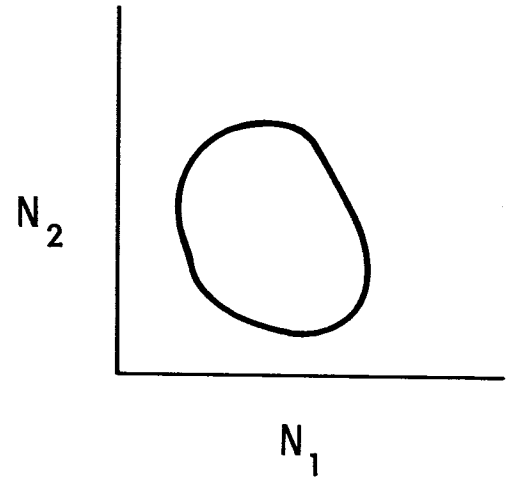

Figure 26.—Periodic behavior of one species, N_1, plotted against another, N_2.

The situation is idealized and I use the word "idealized" and hope that it will be taken in the context of the idealizations made in physics—this is idealized in the Volterra cycle by plotting, let us say, N_1, which is the population of one species, and N_2, that of the other. We have a genuinely periodic behavior (fig. 26) drawn here in some lopsided way to indicate that it is not a simple oscillation. The question is, So what? Here we begin to entertain the question of thinking more broadly about ecological questions. One which Dr. Levins mentioned is this: Suppose that even on a very simplified theory of what ecology is all about—and without apologizing for the theory, just taking it as something to think about, to play with—we may still ask: Given a large number of interacting populations, what may we discover?

Dr. Levins pointed out the very important fact that this problem is really very difficult mathematically. This problem of two species is already serious—this Volterra oscillation is not just a linear oscillation. There is no solution in closed form in terms of simple functions. How can we hope to understand, let us say, 10^8 or 10^{10} species? Let us think about the whole biosphere. As long as we are speculating, let us speculate rather broadly. The answer is that we may indeed to so, at least within the context of the Volterra theory, and I think this is a commentary not so much on the Volterra theory but on the method by which we may think about complicated systems.

Let me illustrate the point further in physics before making it with regard to this idealized model of ecology. I think that we know more about 10^{23} atoms in a bottle of gas than we know about the Moon-Earth-Sun system. Why is that? It is because we have a new way of both measuring and thinking about 10^{23} atoms, and that way has come to be called thermodynamics or, more properly, statistical mechanics.

If we introduce a new concept, temperature, that is very interesting. Temperature does not tell us what any one single molecule is doing. It does not tell what the collisions are like, at least not in any detail. It is a physical comment on the structure of the entire system. Therefore, I will just naively ask this—and this is perhaps trying to pour new wine into an old bottle and may not be a good idea except to illustrate a point of methodology—Is there an analog for temperature for the ecosystem? Let us take 2n species in interaction, population numbers N_1, $N_2 \ldots N_{2n}$. The number of species I will take, peculiarly, to be even. There is a difference between an even number and an odd number. I am sorry—this may or may not have any genuine meaning, I do not know—but an even system has essentially what might be called a conservative property or a kind of cyclical property that the elementary two-species system has also.

What can be said about the Volterra dynamics is this: there is something that is conserved, even though the motion be very complicated. I will write down briefly what the something is without explaining exactly what lies in back of it, and then we will continue from there. What is conserved is this:

$$G = \Sigma q_i \beta_i (e^{v_i} - v_i) \text{ where } v_i = \log \frac{N_i}{q_i}$$

Here β_i is a species-specific parameter, and q_i is a special population level of the i'th species at which the populations can all be static. The q values are independent of the way the system is started up. The v stands for the logarithm—of all things—the logarithm of the population number divided by this very particular q value in which a steady state is possible. This quantity is constant. It is just a mathematical property of the Volterra system.

The reason that I represented the constant (which I will call G) in this particular way is this: If we study the system in this N_1 - N_2 plane, we may learn some things which will be more or less instructive. We can learn much more, interestingly enough, by studying the variable v instead of the variable N. Why is this? Let us imagine a space of a large number of variables, v_1, v_2, v_3 and so on; this is some kind of Cartesian space to help us think about the progression of the whole motion of the system. Then we take one point where the unfolding of the motion corresponds to the motion of this point. We study the motion in this v-configuration space instead of the N-space because if we take many points so that we make a whole fluid, the volume of the fluid is conserved. The fluid in this v-space is incompressible. In this N-space it is not. Thus, there is another conservation law. This is the conservation of volume in v-space; technically, the physicists call this a phase space where volume is conserved in such a way as this.

Now to the Gibbs theory. What we call statistical mechanics, first of all, really has nothing to do with mechanics. The Gibbs theory is a statistical theory of differential equations, and it is exactly at that point that it may possibly have some interest and use for biological problems. The three things needed to engage in statistical mechanics by the Gibbs theory are the following: (1) We must have this conservation law of volume in v-space, Liouville's theorem; (2) We must know <u>something</u>—for example, that G is constant; (3) We must be very, very ignorant otherwise. We must not know too much. Interestingly at this point we have a kind of embarrassment in biology. I have an awful feeling that we know too much and are trying to make too much use of what we know.

ATWOOD: In order to understand what is being conserved, may I ask what would be the consequence if either of the conservation laws were violated?

KERNER: The consequence for what or whom?

ATWOOD: For us.

KERNER: We would not be able to engage in Gibbsian statistical mechanics. We would not be able to analyze, to introduce a concept for, this complicated system analogous to temperature. The point is that we have to fix things up. If we begin with some very complicated differential equations, then when we use the variable N and consider the fluid in N space, we do not have the Liouville theory—we do not have the conservation of this fluid volume in phase space. In practice, the idea of considering many points means that, in the absence of real knowledge about what the system is doing beyond this little bit, we say the system could have been started up in any one of a number of ways. In other words, we make a large number of mental copies of the one system that is going on, subject to the one restriction that G is constant. The system is extremely complicated; we do not want to get in the bind of saying that each of 10^{23}

variables can be specified initially. We would not have enough paper and ink in the entire universe to even write down such initial data.

ENGELBERG: Dr. Kerner, can you give any simple biological significance for the G that is conserved?

KERNER: No, I am afraid I cannot.

ENGELBERG: It must have some relation to the variables in the basic equations. In other words, what is invariant in the initial equations such that when transformed into phase space it will yield an invariant G?

KERNER: It is something not immediately apparent. The conservation of G is intrinsic in the Volterra equations after a reduction.

POLLARD: Is it not fundamentally true that the probability has to be one?

KERNER: The existence of G?

POLLARD: Does it not mean the whole thing has to be there somewhere?

KERNER: Not exactly. This is only a statement which I would like to say is crudely analogous to the statement of energy conservation in physics. Even though we do not know what a large number of particles are doing under certain general hypotheses, we know that the energy is conserved and we can write down the energy, and the situation is similar here. This thing, G as described, is conserved.

ATWOOD: Could you tell us in somewhat more verbal form what is conserved?

LEVINS: What will make G larger?

KERNER: Let us write this down in the original language. We will write G this way:

$$\Sigma\, q_i \beta_i \frac{N_i}{q_i} - \log \frac{N_i}{q_i} .$$

ATWOOD: What is N?

KERNER: N is the original population number measured in units of q, which represents a point. The value of q for each of the species represents a value which, if attained simultaneously for all the species, will mean that these species did not fluctuate at all but that they have certain constant values.

LEIGH: Would it be illegal to introduce at this point the fact that we prove that q would also represent the average value of the population size for the species in question over a long period of time?

KERNER: That would be fine, but you stole my thunder. That is something that remains to be proven.

LEIGH: I guess it remains as is. If it is convenient to you to disregard it, go ahead.

KERNER: Let us do away with the thunder. The unit q has the following significance. Let me try to draw some pictures. I do not want to become involved in a lot of formal details, but will simply schematize what the principle is. Suppose we had a complicated Volterra system and then we were clever enough to assay the population of one species, say No. 27, as a function of time; under the hypothesis that the system is indeed complicated, we find a very complicated behavior.

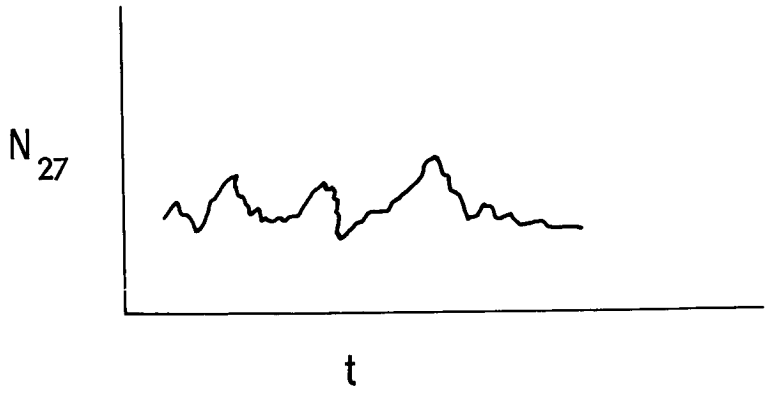

Figure 27.—Assay of the population of one species as a function of time.

If we look at this (fig. 27) as being a single segment of a very complicated system we say, "Forget it. This is noise." But that is the point—it is noise. But we want to understand something about what the noise tells us in the same sense that we want to understand what the noise is that describes the trajectory of a Brownian particle. It tells us a very great deal. For example, it is a temperature indicator. We can understand what the temperature of a physical system is by looking at the Brownian trajectory.

The very complicated workings of the large systems are reflected in the statistical properties of this time sequence, and the whole essence of statistical mechanics is not to make statements about what any single variable is doing but only statements about what is happening in the mean over long periods of time. For example, what is the average population level? What is the mean distance between where this curve hits this horizontal line? How big are these upward peaks in the mean and these lower peaks in the mean?

These are the questions that statistical mechanics has the power to answer. Insofar as we have very complicated systems to observe in biology, it may be that this is a type of question which we shall be entertaining. And it may be that we should be entertaining a type of analysis of the statistical behavior of one variable in time.

LEVINS: Considering the past and present history of biology, if this type of analysis is correct and we start playing around with G, in 50 years from now we will be tossing G around, the question of the size G, in the same way we here are talking about the operon or any of the other abstractions. It is not necessarily any more abstract, but less familiar; therefore, we do not have any intuitive feeling for it yet.

KERNER: If we look back in the history of physics, we will see that in about the 17th and 18th centuries people did not understand what energy was. They were all involved in the difference between MV and $1/2\ MV^2$.

ATWOOD: They had a value judgment that energy is good and matter is bad.

KERNER: So be it. The point is, Why are we so happy with energy? We have become conditioned; we have learned it has a valuable property, that it is conserved. That is why it is so valuable, among other reasons. Thus, I think Dr. Levins' point is very good. I cannot say any more than is here. This is what it is. To ask me for something more is what I cannot say.

LEIGH: I think I may be able to explain some aspects of that G. It has no biological significance as yet, but there are two things about it which might be helpful. We start with a set of fairly

complicated differential equations. Written in the most complicated possible form, they appear as

$$\frac{dv_i}{dt} = \sum_{r=1}^{n} a_{iv} \frac{\partial G}{\partial v_r}$$

The G is that G which everybody distrusts and hates so much. In general, we are interested only in the statistical properties of the solutions to this equation. From the mathematics of the problem it so happens that the only way we can get at this is by averaging in some sense over the set of all possible v_i consistent with a given value of G.

ATWOOD: Will you redefine, please, the symbolic representation?

LEIGH: In the equation $v_i = \log N_i/q_i$, where N_i is the population size of species i and q_i is the average over a long period of time of the population size of species i. This last is a theorem which has to be proved, and I am assuming it beforehand for I will not have time to prove it.

The assumptions concerning the meaning of the a_i's appear most blatantly when we write the Volterra equations in terms of the masses of the species involved. (Here X_i is the mass of species i.)

$$\frac{dX_i}{dt} = e_i X_i + \sum_{i=1}^{r} a_{ir} X_i X_r$$

The rate of change of species i is assumed equal to $e_i X_i$, where e_i would be the logarithmic rate of increase of species i, if everything else were moved away (thus e_i would be negative for carnivores), plus a term $a_{ir}X_iX_r$ summed over r, representing the effect of the other species of the community on the rate of change of species i. We have assumed that the effect of species r on species i is proportional to the number of meetings between the two and that, just as in a gas, the number of meetings between the two is proportional to the product of their population sizes. Worst of all we assume, quite without shame, that the a_{ir}'s form a skew-symmetric matrix $a_{ir} = -a_{ri}$, which corresponds to the biological assumption that when one animal eats another the total weight of the prey is converted into weight of the predator, which of course is quite wrong. I will shortly explain how we can hope to use this assumption and still obtain meaningful results: things are not quite as bad as they may look.

We get our equation in G from the Volterra equations by a change of variables. From this equation the corresponding expression for G is constant in time. The requirement that G be constant comes in when we want to evaluate the probability, for example, that v_i is less than some fixed number. To do this we must consider all possible values of v consistent with a given fixed value of G. If we try to average over the entire phase space, our integrals would not converge, and we would not obtain any answers; whereas, if we average over a "shell of constant G," we can get an answer that seems to make some sense. This G is thus a mathematical necessity. As far as I know, it has no particular biological significance; there is no justification for its conservation other than the fact that it has to be invoked in order to prosecute this purely mathematical method of analyzing complex systems.

ENGELBERG: Might it not be better just to take a derivative of v_i? Then on the left-hand side we will have $(1/N)dN/dt$, which is merely the specific growth rate—that is, dN/dt divided by N rather than dN/dt.

LEIGH: In other words, you want me to write this equation in terms of d log X_i/d?

ENGELBERG: Write it in terms of the N's instead of the v's, because it is difficult to keep visualizing what the v's are.

LEIGH: The reason for changing variables from N's or X's to v's is that by doing so we have transformed our equation into a form subject to a method of analysis of fairly great power—a method which, applied to the system of equations

$$\frac{dy_i}{dt} = \sum_{s=1} a_{is} y_s = \sum_{s=1} a_{is} \frac{\partial}{\partial y_s} \sum_{k=1} \tfrac{1}{2} y_k^2$$

yields the shortest proof I know of the fact that a superposition of a large number of sinusoidal vibrations is Gaussianly distributed over time. The power of this method, incidentally, rests in the fact that it is concerned only with the statistical properties of the solutions of the systems of equations to which it is applied, and the method becomes useful only as the number of equations involved becomes large. But all this does not alter the fact that the equation closest to the biological meaning of the situation is that involving the mass of species i:

$$\frac{dX_i}{dt} = e_i X_i - \sum_s a_{is} X_i X_s$$

Now I will justify the assumption of the skew-symmetry of the a_{is}. The remark I wanted to make was that in statistical physics we recover essentially the same results if we assume a conservative system free of friction and completely isolated from its environment as we do if we assume that the particles are subject not only to a friction but also to a random series of "environmental shocks," as in the Langevin equation

$$\frac{dv}{dt} = - kv + F'$$

where v is velocity of the Brownian particle, k is a coefficient of friction, and F' is sort of a random force term.

The assumption that $a_{is} = -a_{si}$ means that the system of equations is conservative in some sense (in particular, this assumption enables G to be conserved). If we alter the assumptions about the a_{is} in a biologically reasonable manner (such an alteration would involve setting a_{ii} to be strictly less than zero, which represents the harmful effects on a given individual due to crowding by members of one's own species), we find that G generally tends to decrease over the course of time, the system of equations becomes dissipative, and, appropriately in this case, population fluctuations tend to damp out over the course of time. The analogy with statistical physics leads us to hope that adding an appropriate force term corresponding to environmental fluctuations to our otherwise more correct dissipative equations leads us to results similar to those of the "statistical" theory of our original conservative equations. In this view, the Volterra equations are in the end devoid of any biological significance, but to some extent they produce the same answers as equations which may mean something and, what is important, may be easier to work with than the more meaningful equations.

HOFFMAN: I think I might have missed the explanation for wanting a minimum of v. Did you say there was a minimum of the v's?

LEIGH: A minimum of v's?

HOFFMAN: You did not say that?

LEIGH: No.

HOFFMAN: In the Volterra equation that you wrote out, the G's are constant; right?

LEIGH: I misunderstood the question. The point is that G is independent of time, but this does not preclude its depending on the v's.

HOFFMAN: I thought you said——

LEIGH: I think at one point this may have been in. I wanted to evaluate the probability, say, that v_i was less than some fixed number. Was that the point?

HOFFMAN: Yes, I see.

LEIGH: In other words, this was something we wanted to evaluate.

KERNER: Two things are conserved. One is G, which we should think of roughly by physical analogy as the energy. It is the ecological analog of energy. The other is volume in phase space. This is a purely geometrical object that is also conserved.

MANILOFF: Is G a function of all the v's and of t?

KERNER: The v's depend on time but G does not.

MANILOFF: When written as a constant, does that mean it is a constant with respect to t?

KERNER: That is correct.

MANILOFF: But not with respect to v?

KERNER: It is a function of the v's. The point is that no matter how the v's change in time G has to remain fixed, just as when the velocities of a whole group of particles change the sum of kinetic and potential energy does not change, even though the velocities are all changing. Similarly the Volterra v's are all changing, but this quantity G is not. It is constant in time.

POLLARD: Mr. Chairman, may I remind you of Gerald Zacharias' dictum that if one knows what he is talking about we can explain it to others. Could I suggest that we know what these things are about here? At the present time, I think this is simply verbiage. It is formal and nice, but how does it relate to reality?

LEIGH: One theorem of importance that emerges from the theory is the following: If we measure the total interaction between the species of a community by $\Sigma|a_{is}|$, then in some sense the stability of the community is maximized for a given degree of interaction by setting all the $|a_{is}|$ equal. In particular, setting the $|a_{is}|$ equal to each other reduces the frequency of population explosions and population crashes, and it also reduces the chance of a serious perturbation in the numbers of one species causing a dangerous low in the numbers of another.

This theorem is simply the ecologists' law that the stability of a community increases with increase in the number of links in its food web. The molecular biologist knows this theorem also, for he has seen that the multiplication of feedback loops, in the form of feedback inhibitions and induction-repression systems, increases the stability of his bacterial systems. Some idea of this result's importance to the field ecologist can be gained from MacArthur (ref. 89) and Hutchinson (ref. 90).

Other uses lie in the direction of probability distributions for population sizes for the same species at many times and, hopefully with a little elaboration, for many species at the same time. All this is of great interest to the ecologist, for he has some empirical data on these questions he would like to understand.

WESLEY: I like Dr. Kerner's theory.

KERNER: Dr. Pollard, may I ask you if you would accept the picture of an atom as being a little spring with an electron on one end of it and a proton on the other end?

POLLARD: This has nothing to do with atoms. This has to do with populations.

KERNER: This has a great pertinency for what we do with atoms. In both cases we are making models.

POLLARD: What I am concerned with is wherein lies the significance of the Liouville (Ll) system. This I think is obscure. Everything else is all right.

KERNER: I think we were sidetracked, and your point is important. You are asking what is behind this Ll theorem.

POLLARD: Why is there no accumulation of points in this space?

KERNER: The idea of Gibbs was that if we look at the cloud of points, coming back to the main theme now, and provided that the cloud of points has a conserved volume, we have a tool whereby we could associate a given volume in phase space with a probability for finding a point in phase space. In order to conserve probability we must have a conserved volume or else the idea of probability is completely lost. Thus the point of Liouville's theorem (and this is quite crucial to anything having to do with statistical mechanics) is that it gives us a way for associating volumes in space, in phase space, with probabilities for finding the system in that particular volume.

POLLARD: How different is that in an application to biology?

KERNER: That I do not know. At the moment I only know that there is one perhaps interesting system of equations in ecology for which it is true that, provided we work in this space, there is a Liouville theorem. The space is really quite important. We do this by habit in physics now. We work in momentum and coordinate space. Why momentum and coordinate space of all things? Why not just in some velocity and position space? The reason is that we have a Liouville theorem in one but not in the other. This is why canonical variables are so canonical.

LANGELAND: Are you able to define Hamiltonian equations in this system?

KERNER: Yes.

LANGELAND: To me, the theorem is intimately connected with the Hamiltonian equation.

KERNER: This is an important technical point, which I would like to come to in a moment. I think I am running out of time.

ENGELBERG: One more question on your last point, Dr. Kerner. I believe that in statistical mechanics one of the advantages of going to phase space and using canonically conjugate variables is that every volume has the same a priori probability. Is that correct?

KERNER: That can be made as a separate assumption. If we do so we know that probability will then be conserved.

ENGELBERG: Is this true of your system, also?

KERNER: It is true here just as well as in a physical case.

ENGELBERG: Does every volume have the same a priori probability as every other volume?

KERNER: This is a possible way for assigning probability. We know that once a way for assigning probability is chosen, the idea of using probability itself is valid because we have a probability conservation law. If we did not have this, it would be meaningless to say that we have probabilities and they did not all add up to 1.

This discussion of Volterra's ecological dynamics has only touched the surface; the compressed timetable has permitted only some attempt to state the preliminary points. My hope was to sketch the closeness of ecodynamics to classical particle dynamics and, in particular, to indicate that the Gibbs ensemble theory as developed for the latter is also perfectly applicable to the former by showing that typically "thermodynamic" parameters, as temperature and entropy, can indeed be used to characterize the large and complex ecosystem. A summary discussion, with detailed references, can be found in the Annals of the New York Academy of Sciences, volume 96, Mar. 1962, pp. 975-984.

LEVINS: I think I will have to end this now and go on to a different aspect of population biology with Dr. Crow.

CROW: I am substituting for Dr. Sewall Wright, who was invited originally to discuss the subject of population genetics. I want to allude very briefly to some of the general results of population genetic theory and then discuss a few more specific points, considerably more elementary mathematically than we have been talking about for the past few minutes.

As everybody knows, the theory of population genetics is largely the work of three men: Fisher (ref. 91), Haldane (ref. 92), and Wright (ref. 93). Fisher put a great deal together in one statement which says that the rate of increase in fitness of a population is equal to the genetic variance in the fitness of the population at that time. It is a remarkable theorem. It depends on the correct definition of variance. I think almost everybody would expect that the rate at which a population evolves depends on the amount of genetic variability in the population; but that it would be measured by the variance rather than some other measure of variability is not immediately self-evident. Also, the fact that the proper definition of genetic variance is related to conventional statistical measures strikes me as an interesting point. The definition of genetic variance is a least squares definition, and the way all of this fits into the theory of natural selection is one of the high points of Fisher's book.

Some of this has been carried a bit further. Fisher analogizes the concept of fitness with that of entropy in a thermodynamic system, and indeed, there are some parallels. Other parallels with physics are found in some of the developments of population genetics theory. I think this mainly reflects the fact that a population geneticist has to look somewhere for his ideas, and they are more likely to come from classical mechanics than anywhere else.

When one of my colleagues Dr. Motoo Kimura was working on equations dealing with rate of change of gene frequencies, he found a quantity which does not have, to me at least, any obvious biological meaning; but it is a quantity which, when maximized, leads to the equations for gene frequency change. This reminds me of some of the superlative principles in physics, such as the Hamilton principle or that of least action, that when maximized or minimized lead to the equations of motion (ref. 94).

Another analogy with the kind of theory that has been developed in physics has been the stochastic treatment of gene frequency changes. There is a great similarity, in many instances amounting to identity, between diffusion and heat conduction equations and the equations that turn up in evaluating the change in gene frequency under directive and random forces.

I want to talk more specifically about two concrete points. The first is a special case, but I will deal with this rather than with a more general model because I think all of the interesting points come out in this special case. This is an idea that was deduced by Haldene and has to do with what might be called the cost of natural selection (ref. 95). Perhaps it might more precisely be called the cost of keeping up with a changing environment. Suppose I have two genetic strains A and B, or two genotypes. To simplify, assume the organism is haploid. Let the frequency of type A be p and of the alternative type B be 1 - p; the fitnesses of the two types are assumed to be in the ratio $1:e^{-s}$ or, when s is small, roughly 1:1 - s.

To fix ideas, think of it this way: Assume that the environment is continuously changing. Previously B has been the favored type, but because of a change in the environment some genes have been made more favorable and others less favorable. This will make some previously favorable genes detrimental and vice versa. But there is one troublesome consequence—the genes that were previously favored will be the common ones, and the newly favored ones will be rare. The population has the problem, and the cost, of making these common by natural selection.

Therefore, suppose that the A type was previously harmful but is now favorable. We ask how much selection, that is how much selective elimination of alternative types, is required to bring the rare type to a high frequency.

We can write an equation for the rate of change of p as follows:

$$\frac{dp}{dt} = sp(1 - p)$$

I think it will seem self-evident that this equation is at least reasonable, and I do not want to take time to derive it in detail.

If I want to express the total cost to the population during the time that it takes the rare gene to become common, it can be written this way:

$$\text{Cost} = \int_0^\infty s(1 - p)dt$$

This will be an integral of the amount by which the B type is deficient in s, weighted by its frequency and integrated over time. Changing variables and substituting from the first equation, we obtain

$$\text{Cost} = \int_{p_0}^{1} \frac{s(1 - p)}{sp(1 - p)} dp = - \ln p_0$$

The limits of integration change from 0 and infinity to the initial and final frequencies of p, namely p_0 and 1. Especially nice is the fact that the s's cancel out in numerator and denominator, and the total cost is a simple function of the original frequency of type A.

I brought with me some notes on the magnitude of the cost. If p_0 is 10^{-6}, the cost is 14. If p_0 is 10^{-4}, it is 9; if 10^{-2}, it is 5. With diploidy and dominance the value is usually somewhat larger, especially if the newly favored gene is a rare recessive. The part I am interested in pointing out here is that this expression is independent of s, provided that s is small enough that a continuous model is applicable.

ATWOOD: Do you mean provided that p-s is approximately 1-s?

CROW: Yes, and also that I can substitute integration for a summation process. Take the value 14; this means, say, that there must be 14 selective eliminations for each gene substitution of its type. To say it another way, if I would like to substitute one gene every generation, the population must have sufficient reproductive power that it can eliminate 14/15 of the population every generation and still perpetuate itself. I should emphasize that these are selective eliminations and not merely chance deaths; whatever random noise is in the system must be in addition to this. This principle gives some indication of the rate at which a population can make gene substitutions over a period of evolutionary time.

There are many simplifications in this formulation, but I think this principle (which is the kind that Haldane seems particularly capable of discovering) gives some new insights. The cost of a gene substitution depends primarily on its rareness in the first place.

ATWOOD: Then, the initial frequency is equal to the mutation rate?

CROW: Mutation rate together with former selective disadvantage or whatever other factors determined its frequency prior to the time when the gene became favorable—yes.

This also suggests ways in which substitutions can be made at less expense. For example, this tells us that it is far cheaper to substitute a gene that was formerly only slightly deleterious than one which was grossly deleterious.

LEVINS: This means also that even while the gene is deleterious it might be assumed to have mutations to keep up.

CROW: Yes.

LEVINS: Assuming it is completely deleterious, then it is mutation rate.

CROW: If it is completely lethal, it would be the natural log of the mutation rate—or rather the absolute value of this.

LEVINS: The interesting thing is that in a fluctuating environment of this type and also in a model, it can be advantageous to have lethal mutations.

ATWOOD: Provided the lethal finds itself nonlethal in another environment.

CROW: By "lethal," is meant deleterious, not necessarily completely lethal but disadvantageous. Presumably, if a population were behaving in an optimum way, it would somehow devise a mutation rate which gives enough mutants to take advantage of environmental change without at the same time having so many mutants that the population fitness is dangerously lowered.

LEVINS: There are two objections that have been raised to this argument of Haldane. First, the cost calculated in this way is not necessarily a real cost; with the replacement of one gene by another the population size, for instance, does not necessarily go down, the reason being that this is a measure of relative fitness. If, however, a population has saturated its environment, it may not be noticeable at all.

For example, in a rodent population experiment with each litter having five young rats, they have irradiated the young to such an extent that the litter size dropped to three. Nevertheless, the population density did not change at all, so that in a sense the cost of selection is a measure of how much surplus reproductive potential has to be before we can even notice the effect of replacing one gene by another.

CROW: There may be various trick ways of absorbing some of the cost, and one way might be to have an excess litter size, as you suggest.

LEVINS: The second objection is that in more complicated models than the most naive there is a linkage disequilibrium; genes tend to associate together and therefore the death of a single individual may eliminate several deleterious genes at once. Thus, linkage disequilibrium is a way of cutting down on the cost, making the same elimination of multiple genes.

CROW: I would agree; linkage under some circumstances might decrease the cost. We can think of other detailed ways of cutting the cost, and a complete theory would include this; nevertheless, I think the simplest case that I gave presents in the most direct way the principle that I am after.

ATWOOD: You mean, of course, that the population size does not have to change at all if this keeps going on.

CROW: Right.

ATWOOD: That the population size is determined by what Schmalhausen called "nonselective elimination"?

CROW: Within the framework, yes. We might say, for example, that an organism producing a million progeny of which only one on the average survives is not necessarily having much selective elimination. This is all built into the definition of s. If there is a great amount of random elimination, s is correspondingly smaller; that is, if most of the deaths are random, there is not much selective distinction between one genotype and another.

ATWOOD: If most of the deaths are random this still works.

CROW: Yes, that is right. That is what I am trying to say. The Haldane principle is still true. Random deaths may change the value of s, but this quantity does not appear in the final equation.

Let me repeat what I said at the beginning. This kind of statement gives an idea of how much selection measured in terms of eliminations is required for a population to keep up with changes in the environment by making gene replacements.

Another principle of the same kind, also due to Haldane (ref. 96), tells what the cost is to the population of having mutation acting on it all the time. How can we quantify this effect? We can if it is measured in terms of genetic eliminations or reduction in fitness, as in the previous example. If a mutant is very harmful, it is eliminated quickly and affects a small number of individuals; if a mutant is only mildly harmful, it is eliminated slowly and affects a large number of individuals. I think it is not unreasonable that these effects will cancel each other out. In fact, if the right units of measurement are chosen, they cancel exactly, and each mutant has the same impact on the population. The effect of mutation on the population will be related to the mutation rate and not to the harmfulness of the particular mutant.

We can go further than this and ask the influence of other factors on this situation, and this is what one of my colleagues, Kimura, and I have been doing recently (ref. 97). Those of you who know me realize that the mathematics of this are beyond my powers and that this part of the study should be attributed to Kimura. The results are shown in figure 28.

I want to make sure you understand what I am talking about. The ordinate (fig. 28) is the proportion by which the average population fitness is decreased by the recurrence of mutation. This will be equal to the mutation rate for a recessive gene in a large population. It will be twice the mutation rate for a dominant or partially dominant gene, since two recessive genes are necessary to cause an elimination and only one for a dominant.

What happens if the population is finite? We can work this out. It is clear that as the population size becomes smaller the load will rise because the population is subject to random fluctuations in gene frequency, and these fluctuations will in general decrease the fitness.

What is not intuitively clear, at least to me, is the shape of the curve for different values of s, the selective disadvantage of the mutant gene. It might be thought that the Haldane principle would be true irrespective of the population size and of any selective disadvantage, or there might be a curve such as any one of those in figure 28 but the same for all s values.

The point I am interested in emphasizing is that there is a large range of population sizes, from a few hundred to a few thousand, where a mutant with 0.1-percent selective disadvantage causes a greater reduction of fitness in the population than one with 1-percent disadvantage. Since I suspect that many mutants are in this range of selective values and that many populations have this range of effective number, this may be a rather significant observation. I am using effective population number in the sense that it is used by Sewall Wright (ref. 98).

ATWOOD: It is certainly against intuition. I think you ought to go through it again, because it is so much against intuition it almost defies it. You say the one with the least selective advantage has the most effect?

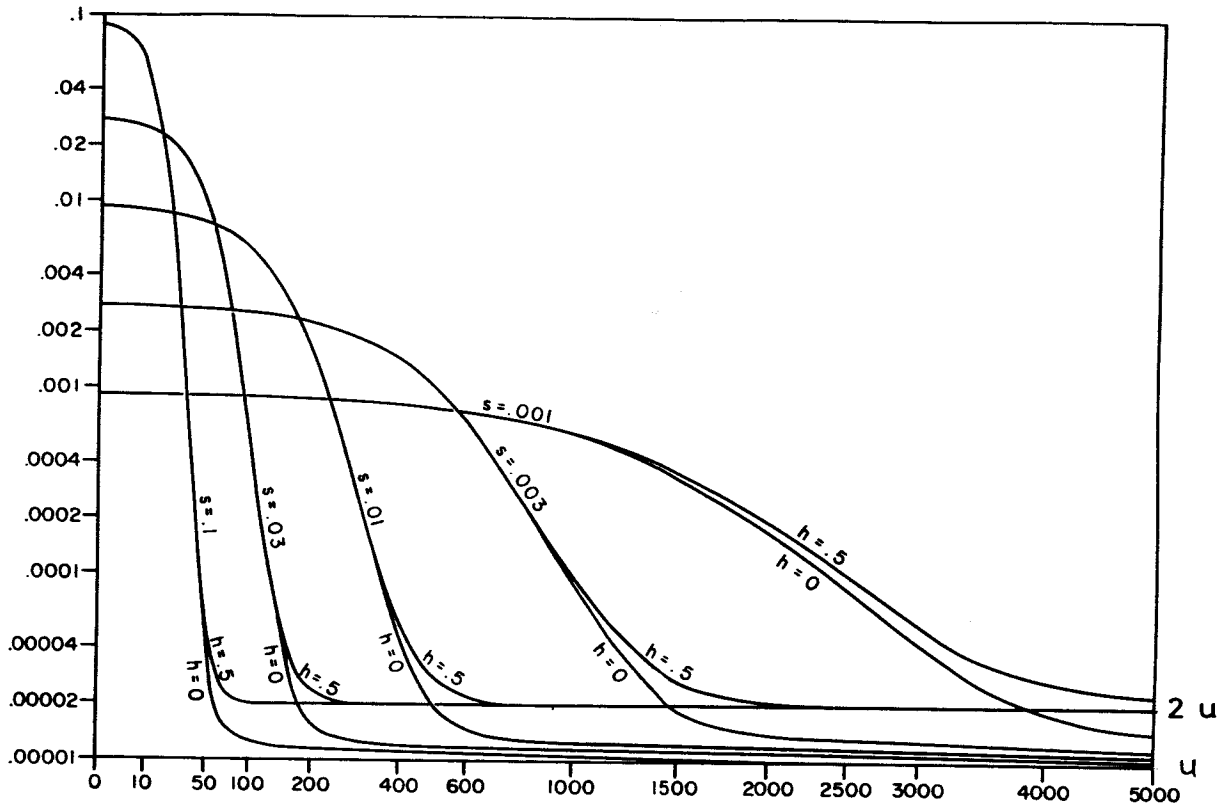

Figure 28.—The mutation load (ordinate) as a function of the effective population number (abscissa) for various values of the selective disadvantage of the mutant homozygote, s. The line corresponding to h = 0 corresponds to a completely recessive mutant; h = 0.5 is for a mutant that has no dominance. The forward mutation rate is assumed to be 10^{-5}; the reverse rate is 10^{-6}. (Ref. 98.)

CROW: Yes. The only thing I can say is that there is going to be more random drift for a wild mutant than for a drastic one. Somehow the magnitude of this drift effect is more than sufficient to overbalance the greater individual effect of a drastic mutant, in certain sizes of population.

ATWOOD: You are assuming mutation rates similar to that of the more or less deleterious mutants?

CROW: I am assuming that. We are concerned with equilibrium between the occurrence of new mutants and their gradual elimination. In an infinite population the load is independent of the value of s. As the population becomes finite, then the fate of a mutant is not strictly deterministic any longer, and the greater random element of this seemingly has this consequence.

I will tell you how this graph was obtained. I could not go through it now, and I do not think you would want to take the time if I could. We take the diffusion equations of Wright that, when solved for the steady state, give the distribution of gene frequencies, and we integrate them. This is difficult because of the complexity of the equations and because of the troublesome borders at 0 and 1.

ATWOOD: It is not impossible to accept. We have the boundary condition of the extinction of that gene, that is the point; and if it were not for that, it would correspond to intuition. Thus, even the area under this one with the 0.01 is still less.

CROW: Yes. I would not be making such a point of this if it were obvious—obvious to me, at least.

QUIMBY: Dr. Crow, is there any way of testing this intuition by actually seeing what happens to real populations?

CROW: This, I do not know. I think you have asked one of the most troublesome questions in the theory of population genetics. Contrary to the situation in many other areas of applied mathematics, I am far surer of the assumptions here than I am of any opportunity for testing the numerical consequences of the assumptions. The assumptions are Mendelian inheritance, population sizes, fitnesses, and other things that are fairly self-evident. From these a conclusion

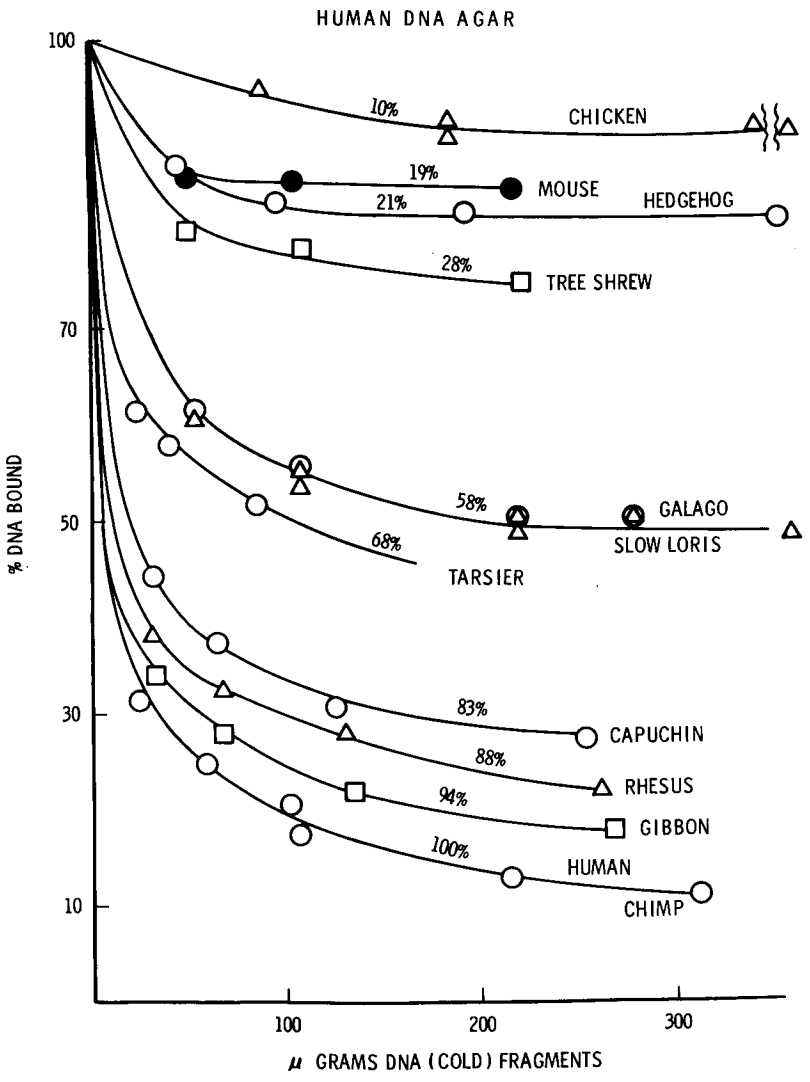

Figure 29.—Competition of various nonradioactive DNA fragments with fragments of radioactive human DNA for sites on human DNA embedded in agar. (After Hoyer, personal communication).

is arrived at deductively; therefore, I am not inclined to urge anyone to try to test particular cases. Does this seem too arrogant?

ROBERTS: Could I put some numbers on the board for a minute? I am not sure whether this is the answer to that particular question, but it is the answer to some. With the DNA agar column, let us measure how many genes two DNA's have in common. Hoyer* has been doing this, and he has tested man against man—we call that 100 percent in common. Testing against the Rhesus monkey, he obtains 88 percent; trees, 28 percent; any mammal, 20 percent; birds, 10 percent; fish, 5 percent; bacteria, 0 percent. Figure 29 shows some results that Hoyer has obtained with a series of animals.

PITTENDRIGH: These are a self-inconsistent set of statements. Any mammal includes armadillo, monkey, and man.

ROBERTS: Twenty percent is the lowest value for mammals, as in mouse, rat.

ATWOOD: I did not think the armadillo is any closer to us than the rat is.

ROBERTS: If we look on these evolutionary trees, it is.

CROW: These are percentages of genes in common, by a criterion of DNA "hybridization"?

ROBERTS: Yes.

CROW: What is it that is 20?

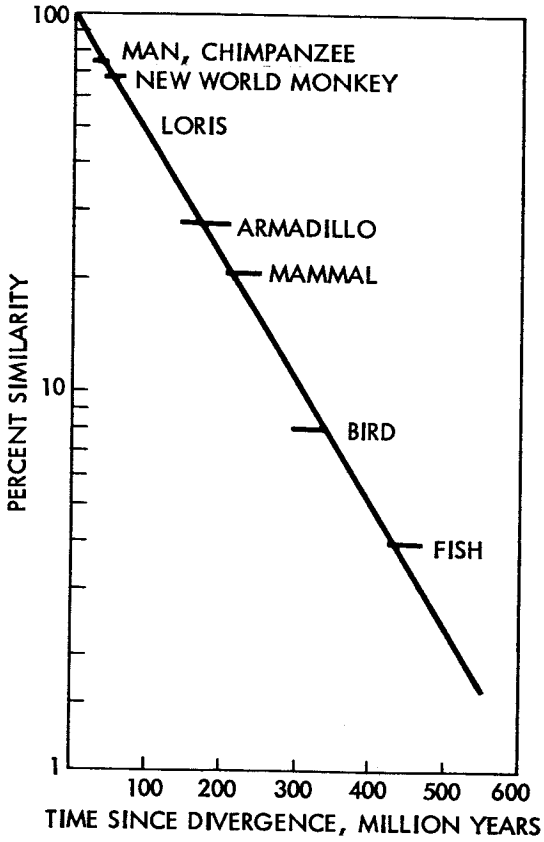

Figure 30.—Similarity of different DNA's to the DNA of the Rhesus monkey plotted against the time since the species diverged.

ROBERTS: Any mammal that we try gives at least 20. These are higher. We can look at the evolutionary tree and try to guess at what time these split off. If we do that and then plot man at 100 percent—and make this a log scale—they all fall nicely on the curve $100\ C^{-T/10^8}$ years (fig. 30). If we test man's genes on mouse DNA, that will select 20 percent of them. If we check those 20 percent on calf or any other mammal, they are the same.

CROW: Is this done by some kind of procedure in which you embed DNA in a column of some sort, pass another DNA through it, and then look for homology?

ROBERTS: Yes.

CROW: What size are the pieces?

ROBERTS: The pieces used to hang up in the column have to be big, and the pieces that are passed over the column have to be small to get into the agar.

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*B. H. Hoyer, personal communication.

ATWOOD: Just to preserve the Fremont-Smith criterion of the meeting, what did Dr. Crow say that reminded you of DNA?

PITTENDRIGH: Or another way of putting it is, what is the cost of fish?

ROBERTS: He said there was no way to test some of these concepts of how quickly genes disappeared from a population. The calculations of population genetics will tell how quickly genes disappear; there is no way to test these calculations. Well, here is a way to test them.

ATWOOD: All right, except that you cannot use your way to really test his way. One is the short run, and one is the long run.

CROW: I am talking about a much shorter time than Dr. Roberts is.

SAGER: I am sorry, but I do not think it should be dismissed quite so summarily.

ATWOOD: It is not dismissed; it is lovely.

SAGER: The hybridization method can be used to look for 0.1 percent of DNA, or even less than 0.1 percent of DNA—not this particular hybridization method but another. However, in principle, this one could be scaled up, or at least a modification of this method could be scaled up, to look for very few gene differences rather than for tremendous ones.

ROBERTS: Yes, that is a different technique. Hoyer is working on the difference between male and female.

CROW: To put it the other way around, a population geneticist is green with envy at this kind of data.

YCAS: I would like to protest Dr. Sager's suggestion. He is speaking of different things. If we have a generative code in which there is, according to the population genera, no change whatever in the phenotype, we might still completely change the DNA and the population geneticist would never notice it. I think this was well brought out by Dr. Slonimski who mentioned the cytochrome c which is virtually the same and presumably for organisms that have different DNA compositions; thus, what we are measuring could have no relation whatever to what the population geneticist sees.

ROBERTS: But it does.

ATWOOD: Intuition is enough to prove that it has a strong relation to what he is saying, except that the time is very different.

DANIELLI: But your intuition was very upset about the armadillo.

ATWOOD: Twenty-five years ago, I was taught that the edentates were very primitive mammals.

ROBERTS: No, they are very advanced.

ATWOOD: What about the Tubulidentata?

ROBERTS: I do not think they tested that one. The armadillo, I do not know. The green monkey and the Rhesus monkey are just the same according to this. We do have the mouse points. Hoyer ran through the whole sequence testing with mouse DNA.

PITTENDRIGH: If the same translator makes a translation of Proust against a translation of Chaucer, we are going to get essentially 100-percent mating.

MOROWITZ: I do not see the point.

PITTENDRIGH: We are using a language. We can have enormously different meanings, while if we dissect the message down to words——

CROW: Let me state that this is all very exciting and I would be interested in asking questions about just how much identity of nucleotide sequence there must be in order for a homology to occur in a system like this, but I really think it is difficult to put that and what I have been trying to talk about together in the same time framework. The way to test what I am trying to talk about would be to say whether we could ever take a population of finite size, selected as intensively as we are able to, not permit any migrants, and arrive at a fitness higher than a certain level predicted by the equations that I have been talking about.

QUIMBY: Dr. Crow, you had speculated on two population sizes. Did you have any particular species in mind?

CROW: This theory would apply to any species if the effective population number were known.

QUIMBY: Are they arbitrary numbers?

CROW: They are arbitrary numbers, but I suspect that in many species the effective population number is 10 000 or less, perhaps considerably less. The effective number may be considerably less than the census number, as Wright has frequently pointed out.

LEVINS: I think we come, then, to the whole question of genetic load. According to Fisher's theorem, the fitness of a population increases proportionally to its genetic variants and reaches a gene frequency that maximizes the fitness. Under these assumptions, then, anything that changes the gene frequency is reducing fitness, and mutation is introduced into this framework as something that displaces the gene frequency from the optimal value. That is why the term "load" was introduced, and it applied to negative effects.

When we allow the environment to vary peculiar things happen. First, the average gene frequency in a varying environment is not the average that maximized the fitness for the average environment. As a result, the average gene frequency under selection in a varying environment is not optimal; in fact, fitness can be improved by mutation toward the less favored allele. I will show the less favored allele as the one deficient in the population.

A second effect of mutation is to increase the correlation between the present gene frequency and the present environment by damping out the effects of the response to selection toward past environments; thus, fitness is improved in this way also and may, in addition, affect the variant. Instead of talking about negative genetic load, if we want to keep that framework of reference, the point is that once we introduce a varying environment a variety of new factors must be considered and thus bring us to the load question.

CROW: I do not disagree. It does not bother me to have a "load" in any of these senses or to have under some circumstances a negative load. I do not mean for "load" to be a loaded word in the sense of implying that it is always bad. What Dr. Levins has in mind depends on fairly definite relationships between mutation rate, cycles of population change, cycles of environment change, et cetera.

ATWOOD: I would like to mention one point to be sure I understand what you mean. If I understood this correctly, it would mean that the higher the inbreeding coefficient, the greater the difference between these two; that is, the more the very deleterious one would have a low load compared to the other one, because it would be eliminated that much faster if we had more inbreeding. Is that correct?

CROW: That is right over a certain range of inbreeding. The curves have to be considered in particular cases. It is the point of intersection of two of the curves that answers your question.

ATWOOD: Let us take a population that has extreme inbreeding, such as a brother-sister regime. Obviously, the one with zero fitness would be eliminated immediately, whereas the one with only 1 in a 1000 could persist.

CROW: It could persist for a long time, yes.

ATWOOD: Thus, the contrast would be increased by inbreeding?

CROW: Yes, until the inbreeding is too intense, as can be seen at the very left of the graph. Perhaps I have not made one point clear; I mean inbreeding caused by a closed population of finite size, not a larger population with nonrandom mating. I am excluding any migration or interpopulation selection. A population of several self-fertilizing lines in which some lines were eliminated, as would be the case if they became homozygous for a lethal, would tend to replace these by expansion of other lines; this would be interpopulation selection in the problem as I have formulated it. My graph refers strictly to what happens within a subpopulation, without any help by migration or intergroup selection.

I would like to say a bit about one other subject. I should like to ask what the advantages and disadvantages are, from the evolutionary standpoint, of sexual and asexual reproduction. I am following, in essence, the methods of viewing the problem that were introduced by Muller (refs. 99 and 100).

Suppose there is an asexual population of genotype A. Now suppose that a mutant B arises that is an improvement over the old type A. Then B will increase and finally replace A in the population. But suppose another beneficial mutant C had occurred at approximately the same time as B. Mutant strains B and C will compete with each other; each will slow down the increase of the other, and finally the best will win out. However, if a C mutant occurs in an individual that already carries mutant B, then everything is fine because both favorable traits can be incorporated in the same individual. If there are a succession of favorable mutants, an asexual system has a considerable problem because it cannot add a second mutant unless it occurs in an individual that already has the first. Thus the average interval between successful mutants (counting only those that are beneficial and ultimately incorporated into the population) will be the time g between the occurrence of a mutant and the occurrence of a new mutant in a descendant of the first mutant.

In a sexual population, on the other hand, as many mutants as would normally occur in this time can be incorporated. If N is the population number, u is the mutation rate (counting only favorable mutants), and g is the same time interval as before (most conveniently counted in generations), the number of mutants that will ultimately be incorporated and which occur in this period will be Nug. Thus the ratio of gene substitution in sexual and asexual populations is Nug. The value of g is obtained by integrating the population number of mutants and asking how long it is, on the average, until there have been enough individuals to permit one mutant. If s is the selective advantage of the mutant, then I have worked out that g is given by the following:

$$g = \frac{1}{s} \ln[(N+1)\exp(s/uN) - N]$$

For $s = 0.01$, $u = 10^{-8}$, and $N = 10^8$ the ratio Nug is 1380. For $N = 10^6$ the ratio is 14.4, and for $N = 10^4$ it is 1.1. Thus, the smaller the population, the less relative advantage of being sexual.

It might be thought that the advantage of sexual reproduction is not so much to put together different mutants, all of which are favorable and favorable in various combinations, but to put together mutants unfavorable by themselves but beneficial in combination. However, I want to argue that a sexual system is not best for this circumstance. The story is the same in haploids as in diploids; thus, let us consider a haploid model for simplicity. Suppose that the genotype ab is the existing type. The single mutant types Ab and aB are both unfavorable, but the combination AB is even better than ab. In an asexual population, the population has to

wait only for the double mutant AB to happen, whereupon this type will take over. The same genotype will occur on the average with the same frequency in a sexual population, but it will not be incorporated. This is because an AB individual mating with the predominant ab type in the population will produce many Ab and aB offspring, unless the advantage of AB is so great, or the two genes are so very closely linked, that this disadvantage can be overcome and the new type incorporated. The system will have two stable equilibria in a sexual population, one with predominantly ab types and the other with mainly AB. But in a sexual population, there is no way to pass from the one stable state to the other. In an asexual population, this takes place directly since there is no barrier to moving to the new equilibrium as soon as an AB type appears.

What I am suggesting is that the advantages of sexual reproduction are primarily for putting together genes which act in an additive manner, as Muller has emphasized, and not for putting together genes whose collective effect is not what would be predicted from their single effects. The ubiquity of sexual reproduction in nature must imply that at the time sex was "invented," the genes primarily responsible for evolution at that time were primarily additive in their relations with other genes; otherwise sex might not have evolved.

LEVINS: This can be very nicely tested by considering those organisms that are only sporadically sexual; we would expect to have a much greater proportion of the epistatic combinations.

CROW: It is related, although I will not say it is exactly the same, to the following: If selection is practiced in an asexual population, a mutant occurs that adds a new level of fitness and then a second mutant occurs. The second mutant can act only in the presence of the first (may very well be a modifier of the first), and therefore this system is likely to build up interdependent sets of genes.

I think I can make it more meaningful by talking about a very concrete example, and also I think it is more meaningful and could be tested best in a system where an organism is being exposed to a totally new environment. An obvious experiment is drug resistance in an organism that has not been previously exposed to the drug. If we use an asexual system, say bacteria of the right strain, a mutant occurs and is incorporated; then a second occurs and is incorporated. The second may well be a modifier of the first and have had no effect on resistance in the absence of the first. If this process continues, we might expect to have selected a resistant strain in which there are a series of mutually interdependent genes producing the resistance. When Cavalli-Sforza (ref. 101) analyzed chloramphenicol resistance in Escherichia coli, this is exactly what he found.

On the other hand, I have selected for polygenic resistance to DDT in Drosophila. On analysis, the resistance is caused by genes that are almost completely additive; there is practically no epistasis at all. The effect of two chromosomes is almost exactly the sum, on the right scale at least, of the individual chromosomes by themselves. This was done by using strains with genetically marked chromosomes (refs. 102 and 103).

FREMONT-SMITH: I thought modern bacteria had become sexual.

CROW: They were not reproducing sexually during the time there was selection for resistance. The sexual process was used later for genetic analysis.

ATWOOD: DDT resistance was reported to break down upon intercrossing of separately selected lines.

CROW: In mine, it did not.

ATWOOD: In King's case, they did (ref. 104).

CROW: One always trusts his own data more than somebody else's, but in my case it strictly was not this way. King's later data did not show this either. I think you are referring to an earlier

stage of his studies. King did an early analysis that showed just what Dr. Atwood said, but there were several other inconsistencies in the results at that time. Later he did another analysis and found the same thing I did; that is, almost complete additivity (ref. 104). I think something was wrong with the early experiment.

ATWOOD: Do you mean resistance was maintained after intercrossing separate lines?

CROW: Right. The F_2 between two resistant strains, or between a resistant and a susceptible strain, was just about the average of the F_1 and the two parent strains. I also did what I think is a more reproducible analysis by extracting individual chromosomes with marker strains in order to know the exact genotype of the recombinant types.

LEVINS: In this question of epistatic interactions, how closely linked the genes are becomes very important. In general, we can maintain equilibrium in a population only if the epistatic genes are closely enough linked so that the evolution of the genotype would involve the accumulation of whole blocks of genes which are together not because of a common origin or because of their nucleic acid structure so much as because of their joint effect in selection, holding them together in blocks and leading to inversion systems and what have been called supergenes.

CROW: Let me make about three final remarks. One is that much of population genetics theory has thus far dealt with single genes or with groups of genes linked according to some simple model of interference. This does not mean that epistasis was not considered, but the interactions were usually of selected types chosen for mathematical manageability. Some of the models chosen by Wright and Fisher are models of cleverness in combining biological realism and mathematical simplicity. But, I suppose that before we understand all this thoroughly, it is going to take more complicated formulae beyond the human mind and, perhaps, will call for more computer analysis.

LEVINS: I just want to disagree with that last point. When we begin to obtain many genes linked together and we cannot handle them as one or two gene models, I think the time comes to switch techniques completely and to consider the chromosome as a continuum with the average effect at some integral along the length of the chromosome up to a certain point.

ATWOOD: This is a Goldschmidt model.

LEVINS: Mathematically, yes.

Dr. Odum wanted to make a few closing remarks similar, perhaps, to Dr. Pollard's summary this afternoon.

ODUM: I have four points. This was not prepared as I was not expecting to make a summary, but perhaps this will add something to what we have heard.

First of all, we all know that orientation is very important. I would suggest that the spectrum of levels of organization might be oriented differently from the conventional vertical arrangement showing the molecular level at the base and the ecological levels at the apex. I would like to suggest a model that is horizontal with the arrangement of levels like an electromagnetic spectrum, or something of that sort, so as to emphasize that levels have different properties but are equally high in the sense of complexity. In other words, I would just like to suggest the possibility—not proved, and others may thoroughly disagree—that ecosystems are not necessarily more complex than cells in terms of scientific approachability. We have all seen that we are not anywhere near understanding the cell; and, as I will show in a minute, we may be already further along in some ways in understanding the ecosystem.

With that in mind, please do not get the idea from my outburst earlier that I have anything at all against applying tools, methods, and approaches that have been so successful at the molecular level to other levels. We have had some very good examples tonight of the usefulness

of extending ways of thinking from cells to populations. All I would say is that that is true or useful. But what else might be true or useful? What concepts, assumptions, orders of magnitude, or other parameters might be additional, new, or replacive as we move from one position on the horizontal spectrum to another?

For instance, let us consider the electromagnetic spectrum analogy. X-rays, of course, would have different properties from the longer light rays, and we would use different instruments in working with these different levels in the spectrum even though, from the physical standpoint, they are all composed of electromagnetic waves. Thus, I would look at the biological spectrum in the same way; they are different properties and, therefore, different approaches at different levels even though the different-sized and different-structured units are all obeying the same physical laws.

Earlier in the conference I made the point that while such principles, such as the laws of thermodynamics, are the same at all levels, the biological structure is vastly different. Thus, unique properties of different levels center around the relationship between structure and function. A kilogram of biomass is a bigger "black box" than a microgram, and it interacts differently with the physical environment to produce quite a different functional system. A forest can greatly regulate and control the physical environment while a single tree cannot; therefore, a forest has different properties from a tree.

I might comment briefly on competition equations. These differential equations have proved instructive in explaining competitive interaction of two rapidly growing species populations in cultures which represent early successional or unstable ecosystems. In complex and more stable ecosystems, many populations are not growing at a given time so that DN over DT will be zero; therefore, the equations are not very useful. Also, the more complex structure of stable systems may isolate the two species that are forced into artificial competition in the limited universe of a culture. We cannot simply add up species growth equations and get a model for the ecosystem. The function of the system as a whole, as well as the function of isolated units, must somehow be the basis for the model.

One important consequence of the presence of many diverse units in a system is the increased ability of the system to regulate itself and dampen the oscillations which we always get when only two or three units are present. An ecosystem can often compensate for temperature and maintain functional homeostasis in the presence of external fluctuations in temperature that would cause marked ups and downs in the metabolism of an isolated individual. We must somehow in the theoretical approach properly evaluate the degree to which the whole is not a sum, or not a linear function, of the parts.

I could go on with many reasons why the properties at ecological levels must be studied at these levels and can be understood only in part by studies at molecular levels, but let me just end with the idea of the "minimum ecological system." I really enjoyed, and was intrigued by the discussion of the "minimum cell." The makeup of this theoretical cell, it seemed to me, depended a great deal on the time it would be expected to survive and on the environment in which it was expected to survive. Dr. Fox's simple microspheres survive quite well in a sterile environment, but are quickly eaten up if there are bacteria in the environment. We may have a parallel in the "minimum ecosystem." If we want a system to function for only one day, then, of course, a small, simple one may do. If we wish to go to Mars, we obviously want one that will operate, survive, and remain relatively stable for a longer time. There must be a curve or family of curves, unknown as yet, that relate survival time to size and diversity. If size, diversity, and stability time (perhaps stability half-life) were plotted on a three-dimensional graph some kind of curvilinear relationship would presumably apply. If we had such a model, we could tell NASA when to shift from a nonregenerative to a regenerative system for space travel.

One final thought. Despite the very small effort that has been devoted to the ecosystem level, we can already come nearer to constructing a "minimum ecosystem" than we can a "minimum cell." This is to say that the ecosystem, when approached at its level, is not a priori more complex or difficult to understand than the cell when approached at its level. Dr. Robert Beyers in a recent paper entitled "Twelve Laboratory Microecosystems" (Ecological Monographs, vol. 33, 1963, pp. 281-306) describes his construction of different kinds of closed systems by allowing the system to select from a large variety of introduced components those components that can operate together as a stable system under a given regime of light and temperature. Some of his closed systems have remained functional at a stable level for several years. Two striking principles emerge from these and other studies: (1) systems must first go through a period of succession before they become stable and (2) the stable systems nearly always contain more than one species in each functional niche—more than one kind of autotroph, for example. The minimum diversity has yet to be determined for a given time-stability requirement under given radiation-temperature conditions imposed from outside the system.

POLLARD: Before we leave, I would like to add one or two things. First, I would like to thank very much the people who have run, under difficult conditions, the ecological side of the conference. Particularly, I would like to thank Dr. Levins for taking over at the last moment and doing a very fine job. I think I would also like to thank our very long-suffering Mrs. Swanson, the stenotypist.

Of course, we have a staff here, and I imagine they must collect these speeches—conference by conference. They probably compare what happened at one conference with another, what this conferee said, and so on. In any event, Dr. Fremont-Smith has quietly regimented us, kept us cheerful, and supplied a condition where we have been able to confer. He has supplied the inner man very well, and he understands the needs of the conferees quite well. We are very much indebted to him. I have known him for a long time; in fact, I first made his acquaintance in this very room. And I may say that I have not yet been at a conference where he was around and doing things which I have not thoroughly enjoyed. This is no exception.

Mrs. Purcell has, of course, another hard job. Where Dr. Fremont-Smith gets us together and looks after the inner man, Mrs. Purcell actually gets us here. She has done that very well, and we thank her for that and for looking after us.

To Miss Gordon, who is to me a newcomer on this scene, I want to extend thanks also. Probably, for all I know she did all the work, and I will not ever be able to find out. At any rate, we would like to give thanks to these three who have really been of great help to us. That is all I want to say.

FREMONT-SMITH: On behalf of the AIBS, I would like to thank all of you for coming, to thank NASA for giving us the chance to make it possible for you to come, and for your tolerance. Also I want to say, naturally, that we are sorry that our scientific studies and communications were disturbed by this very tragic experience that the whole world has suffered.

I hope that those of you who have suggestions for running these kinds of conferences will further communicate with us because these conferences are still an experiment. We do not think we have found the answers. We know it is an ecological system in which we balance one gene against another. We are trying to find the optimal way of doing it, and I am sure we can learn to improve. Please help us in this respect, and again many thanks.

SYNOPSIS

CHAIRMAN: E. C. POLLARD

POLLARD: If I might have your attention! Because Monday is a day of mourning for President Kennedy and because of the very definite way in which President Johnson announced this in his proclamation, the Steering Committee feels that it would not be in order for us to meet Monday; therefore, we will not be holding a meeting on Monday.

It falls to my lot to be an efficient chairman and to pilot this meeting, originally scheduled for tomorrow evening to a rapid conclusion so that, hopefully, we will have, by 9:00 p. m. all the wisdom possible from you.

To speed this up, I am going to ask for suggestions as to the way that we in NASA, and AEC should chart the course for theoretical biology—how it should be supported and what should be done to set about stimulating it.

The following are the suggestions that were made previously as mechanisms by which theoretical biology could be supported. The first one was to hold more conferences such as this with the idea that the areas of theoretical biology would be more specifically outlined and the participants would be invited more specially to take part in those specific subjects.

The second suggestion was that there be theoretical topics chosen, perhaps such very broad ones as theoretical biology, and that there be summer institutes set up for a period of between 6 weeks to 2 months—for example, at Woods Hole, or on the West Coast, or at Pennsylvania State University's summer camp, or at any other suitable place. Perhaps 50 to 150 people could be brought together for either special topics or general topics.

Third, an idea would be to allocate, somewhat like NIH programs, graduate training programs by which money is available for professorships and, separately, fellowships in theoretical biology.

And, last, what sort of previous disciplines would be the ones from which theoretical biologists could be drawn?

I would very much like to hear ideas on any of these four topics and, more especially, on any other topic. These are just to start us off, so will you please shoot at us and give us information.

PITTENDRIGH: I have question number zero: What is theoretical biology?

POLLARD: That is not answerable. Like all these subjects, theoretical biology is what theoretical biologists do, just as biology is what biologists do. If we do not have theoretical biologists, we do not have theoretical biology.

FORRO: Perhaps we should ask this question: How many people would consider themselves to be, at least in the subject area, in the field of theoretical biology?

POLLARD: I do.

PITTENDRIGH: Everybody with self-respect does.

POLLARD: It is not answerable in terms of something that will stand up in the future.

PITTENDRIGH: No, but I think it might be a good thing to pursue. If we were to pursue it, we might try to ask what are the principal questions which separate the life sciences from the physical sciences.

POLLARD: I hope none.

PITTENDRIGH: If there are no such questions, why is it not theoretical science?

DANIELLI: We are not concerned with astrophysics.

TOTTER: The practical problem is that we have to at least prepare a report to give our granting agency.

ODUM: If people will come on their own money, no reports will be needed.

JACOBS: Since we are trying to develop the potential of theoretical biology, a report of this conference would be most helpful. It is possible that a fellowship program as well as summer sessions can be set up. In some of these instances a report would be written; in other instances no report would be required.

DANIELLI: We could combine activities with something on which we do write a report. In other words, we do not write a report covering the whole of the activity of the summer school.

JACOBS: At the end of a summer session, a conference could be held at which the participants discuss their thoughts and ideas. It could be up to them, individually or collectively, to decide on publishing.

POLLARD: Will those who think some aspect of item 2 (summer session) is a worthwhile way to support theoretical biology please raise their hands? (About 14 hands are raised.)

I think, then, that it would be wise for the Planning Committee to say that we would implement the formation of a committee to make a proposal to NASA and to AEC for the formation of one or more summer institutes.

SAGER: I would like to say something heretical. I think that no seminar of longer than a week on the subject of theoretical biology should be held in a place where laboratory and library facilities are not available.

DANIELLI: I support that. I do not think we should meet for more than 8 hours in any place, providing there is a bar. I would also like to suggest that, associated with a summer school, there should be some working parties set up which would meet perhaps two or three times in the course of a year for a day or two, perhaps only five or ten people. What I am thinking of, for example, is the diffusion problem brought up today. This is an area where people could perfectly well leave and think about the variety of problems but meet profitably two or three times a year, perhaps once at the summer institute itself. There are perhaps six such areas, for example, with which small groups could be associated and all come together in a summer institute to discuss how far they had gone with them in general.

POLLARD: Do I hear more comment on that? Dr. Roberts is actually quite knowledgeable on this sort of thing. What is your reaction?

ROBERTS: I think it is very hard to know exactly what a conference like this does. I know that when I talked to Dr. Forro I had some very firm ideas, but I found they may be wrong. Now I want to go back and check them out. That is worth a lot of time.

It is hard to know how much time should be spent at home working and how much time away. If we did the right experiments at home, we could do a year's work in a week. Most of the time we are doing the wrong thing. If we come here and get an idea of what the right thing might be, it is worth a lot.

POLLARD: Dr. Pittendrigh, I was going to ask you on number zero, what do you think?

PITTENDRIGH: I am all for the concerted attack on theoretical biology, and you know it. But the question is whether or not the two major headings of this symposium really covered the field, and I do not suppose for a moment you would suggest they do.

POLLARD: About a tenth of it.

PITTENDRIGH: Let me leave the generality and be a little more specific. If we were to go ahead and have more of these, and perhaps even a summer institute, I do think (and I hope this will not be misinterpreted) that the spectrum of biological, as distinct from immigrant physical, competence ought to be broadened in the group. For instance, I think what is conspicuously lacking is any real competence in the fundamental problems involved in development or in behavior; in other words, there has been something of a de facto decision that the problems can be solved at the macromolecular level.

POLLARD: Not in this conference. This was brought up; actually, it was decided to choose two topics as being what we felt were two quite diverse but hopefully maximum yield areas, and we deliberately chose these topics. We had a feeling if we just assembled everybody who might possibly be interested in theoretical biology, the conference would be too diffused and would not be rewarding such as Dr. Roberts said it was this morning. We had a choice to make, and we may have made it in the wrong direction, but the point is that we are now seeing how we should go.

ODUM: I do not think the two topics chosen were as diverse as they might seem, because discussion of a minimum cell has no real meaning until the minimum environment or the minimum ecological system is also discussed. It is very evident that the definition of a minimum cell will depend on the environment in which it is expected to survive. If the environment contains many readymade organic necessities, the cell can be simple; but if the environment lacks these, the cell must be more complex to survive. Therefore, it seems to me that what Dr. Pittendrigh is saying is that we must consider the whole spectrum.

Whether or not we go into all the details, it is not theoretical biology if we do not consider the whole spectrum; it is just theoretical molecular biology or theoretical ecology, one or the other.

PITTENDRIGH: I did not quite mean that. It seems to me that it would be interesting to know what the geologist wants to use theory for.

ODUM: So that he can do the right experiment or make the right observation, or at least to help him to make better observations or better experiments.

PITTENDRIGH: This is being against sin.

POLLARD: Experimental physics has never wanted to produce theory at all, and since biology is primarily experimental, the generating mechanism is hardly there. In fact, in most physics laboratories that I know anything about, they somewhat resent theory—it is too abstract, too hard to design experiments. They have to get the other fellow to do it and tell him how to do it. It is all based on quantum mechanics and they feel they should not be watching an electron going around. Besides, it is much more fun to build apparatus.

PITTENDRIGH: I think the history of biology has been very different in the fact that it has been loaded with almost too much prematurity. The biologist for a very long time, whether explicitly in these terms or not, has been much concerned with the attempt to explain organization.

POLLARD: Only experimental physicists have explanations. Yes, definitely, they very often are not the ones the theorists would use.

McMULLEN: Is there included in this study of life a study of the theoretical conceptions of the origin of life? We have not touched on biogenesis. Or is biogenesis a subject on which the general consensus is that we do not discuss?

POLLARD: Theoretical biology, in general. Biogenesis has nothing to do with this conference. It will naturally go where it is successful.

McMULLEN: What do you mean by successful—giving a practical result?

POLLARD: No, successful theory. It has nothing to do with practical results.

McMULLEN: How do you define a successful theory?

POLLARD: A self-consistent theory that is right.

DANIELLI: I have not thought about this very much, but it seems to me that there are two main things that we tend to go to theory for. One is the organization of information which was obtained by experiment in order to get the information into a form that can be handled. The other is for prediction. In anything more than quite simple systems, we cannot predict without a theory of the relationships between all the variables that are concerned.

POLLARD: No, there is a third. The third is beauty. This is one of the features about theory that I think is important.

DANIELLI: I think the way in which the Journal of Theoretical Biology came into existence is interesting in this respect. It arose in the minds of about half a dozen people who were really, I think, experimentalists. They felt—we all felt—that the proper way to handle the more difficult problems that we were acquainted with was to use theory as a weapon and, possibly, in the same way that the physicist or theoretical chemist uses theory as a weapon.

Let us say there might be some functions of theoretical work in biology which were not closely similar to the functions that theoretical work has in physics and chemistry, but we just did not know about this. We simply felt that there was a lack and that by establishing a journal we could create a focal point for the development of theoretical thinking.

We were very surprised, actually, by the number of papers submitted, which was about twice what we had expected. Obviously, there were a great many people who, on their own, probably would not have published anything similar as soon had this journal not been established.

Actually, I think this development, which is centered around Dr. Pollard, and which this meeting expresses, represents a different practical approach—the encouragement of theoretical biology, perhaps a more important one. The principal idea on which I feel we should work is that if we are excited about theoretical biology, it does not matter in the least whether or not we can define it. The thing to do is to work on something that we are excited about, and then we get something out of it that is worthwhile, both for ourselves and for other people. The ability to define exactly what we are working on is not, I think, necessarily of great importance. Even if we think we know what we are working on, very often we discover that it is something a little different, and we have to continually reanalyze what we are working on.

So while I sympathize with asking the question, "What is theoretical biology?" in that it leads one to say, "Now, is the piece of work that I am doing really theoretical biology? What is the extent of the contribution which I make by thinking in a particular line?" that is a perfectly legitimate question. But I do not think it is a question to exclude what somebody else is doing.

ODUM: Theory has a function from a different viewpoint that is very important for those of us who teach. Biology is such a tremendous area with so much detail that the only way we can introduce the subject to young people is to have some theory that we can present first. With only a limited time available to excite people about biology, we need some exciting theories that tie things together, and then we can go ahead to the details with more enthusiasm.

GREEN: To my way of thinking, theoretical biology is more a state of mind or a point of view rather than any precise domain. I am tremendously impressed with the fact that, particularly

in biochemistry, there is, shall we say, an obsession with the purely experimental, almost to the point that it is considered extremely poor form to deviate beyond the precise precincts of an experiment. Speculation is almost a dirty word in some quarters.

This state of mind I regard as a disaster for biochemistry, and I am sure there are other fields of biology which are in a similar unhappy state. Somehow it has not been appreciated as well in biochemistry as it has been in physics that it is possible, through the exercise of imagination and solid thinking, to discover things that are implicit in or derivable from the experimental data but which are not obvious, and that this form of activity is worthy in its own right and is a legitimate tactic for solving problems of biology.

I think that all of those in biology who recognize this dimension of science belong to the theoretical biologists. That is how I would put it. Those who consider it as a useless or unnecessary activity obviously would not gravitate to a group of this kind. For this reason, I think theoretical biology would include many diverse types: those who deal with first principles and are mathematically inclined as well as those who think in qualitative terms. This evaluation is probably a far cry from what you had in mind, but that is how I see it.

POLLARD: Mr. Leigh, if we had such conferences as these summer institutes, would you show up?

LEIGH: I would probably prefer to do field work if I could, but that is because I am more of a naturalist. It would depend. I would want to distribute some time equally between both. Perhaps every third summer I would want to go to a theoretical institute if such were available, but I would like to spend the two summers intervening doing field work that had theoretical implications.

POLLARD: Dr. Watts-Tobin, suppose we invited you over here for a summer, would you come to a summer institute?

WATTS-TOBIN: For how long would this be?

POLLARD: You would be a VIP from abroad; you could name the time—6 weeks. Would you come? Now, we are not inviting you, but suppose you received a letter on some school's stationery which said, "This is a summer institute on theoretical biology in Woods Hole," and some assistance, plus a few dollars a day; would you show up?

WATTS-TOBIN: Yes, I think I would like very much to, if I could really spend 6 weeks.

POLLARD: That "if" I do not like. Dr. Bautz, would you show up?

BAUTZ: I think I would like to show up every third year. That is all right, because I would not like to see the same customers every year.

POLLARD: That is right. I think I have already gathered quickly that it is going to be necessary to rotate symposia among several topics so that once every 5 years something is so important that we think we have to go because it is just too exciting to miss.

HOFFMAN: I do not think theoretical biology is really something we can do on a part-time basis and still do it well. A theoretical physicist is a theoretical physicist most of the year, and theoretical biology is nowhere near where theoretical physics is today.

POLLARD: Think of the rate of advance. The rate of advance in theoretical physics is zero.

BAUTZ: Coming back to Dr. Morowitz's suggestion, I think he has a point there. I think I would rather like to go to a summer camp or summer institute because certain people are going rather than because they have certain topics. If I may arrange with somebody to go there, this is much more interesting for me than just to go for a topic. I think it depends more on the persons who are going to be there than on the particular topic.

GARFINKEL: Somehow I seem a bit out of step with everybody else. I am just not that enthusiastic about the idea.

POLLARD: This is the influence of the computer. You have gotten so attached to this computer——

GARFINKEL: No, that is not quite it.

POLLARD: For you, there is no great advantage?

GARFINKEL: It seems to me that being able to think here is more important than being able to talk.

McMULLEN: How will anyone know what you are thinking?

GARFINKEL: It is true a person cannot think in a vacuum, but usually there are a few people with whom it is most useful to exchange information, and this need not be necessarily in a summer institute.

McMULLEN: How do we get them together unless it is a meeting of just two or three friends? There are probably at least a dozen people a person would like to meet at any one time. This needs organization.

GARFINKEL: That has not been my experience. It has been a smaller number, and often enough I can just go and visit them. This may be the result of my method of working, which usually requires close collaboration with a few people at a time.

TOTTER: I wanted to raise a question, which seems to be in the background here, but which I think no one has voiced. To put this on a practical basis, as I understand the misgivings here, we really want to know whether theoretical biology is a more economical way of making advances in biology than the present experimental basis; that if we could add something, we could advance more economically and, therefore, somebody would support this sort of thing.

POLLARD: I have not boiled it down to those terms, and will tell you, I am not going to.

TOTTER: I am not talking about an economical way to learn more biology, to advance the science; I mean economical in terms of time and effort.

POLLARD: All right as long as you are not speaking in terms of dollars.

TOTTER: I think our reason for doing theoretical biology is our reason for doing experimental—because we derive intellectual satisfaction from discovering some order somewhere, but our excuse for doing theoretical biology would be whether or not it is a more economical way to produce or to find the order.

LEIGH: There are some people who would feel justified in working in theoretical biology if pure mathematical problems of interest emerged from the field, and they would feel justified in working on these mathematical problems even if these problems turned out to have no biological significance in the end.

POLLARD: Fair enough. Those people should be encouraged.

LEVINS: I would suggest that this emphasis on theoretical biology as a separate entity is a transitory historical phenomenon: first, as a corrective against the narrow empiricism that has dominated science, especially in the United States, in the past, and, second, as a corrective against the increasing specialization, the move into interdisciplinary activity. Third, I think it is science becoming self-conscious of the strategy of science instead of developing spontaneously. We are insisting, then, on the right to think, but I do not think we want to insist on too great a separation between theoretical and experimental activity. These are phases of

the activity of the same individual. They also represent to some extent the division of labor between individuals.

We also feel that there are certain common problems that make it possible for a theoretical molecular biologist to say something relevant to the geneticist, not simply because he may be a bright fellow. I think also what we have in common is the concern with complex systems. We think there are certain properties of complex systems per se that allow the transfer of ideas from one field to another, which means that part of our work has to be devoted to the analysis of complexity and to the stimulation of some mathematical research that will service this instead of living a parasitic existence on the mathematics that was developed solely for physics.

This means that we have to develop along the lines of encouraging a mathematics appropriate to biology and to complex systems and encouraging communication between the different branches of biology so that eventually we will not have to label every idea we have and say, "Look, this is theoretical."

QUIMBY: Very good.

KERNER: I would like to add a seconding remark to these and that is, it is quite astonishing that in the entire list of participants, there seems to be no one who is, properly speaking, a mathematician, and it seems to me almost a contradiction in terms to talk about theoretical science without its genuine mathematical aspects. I would like to suggest, for whatever it may be worth, that the purely mathematical side of the subject, coming from our mathematical colleagues, is something that in biology we can take good advantage of.

McMULLEN: Dr. Friedenberg, are you a mathematician?

FRIEDENBERG: Yes, I am, but only in the sense that I enjoy using this tool in developing a biological model as analogous to the theoretical physicist. A question was raised in the interim whether the biological models we are discussing would be bona fide models to the theoretical physicist. I would not think so! The approach to building any model should involve: (1) a highly idealized mathematical treatment independent of the physical aspects of the problem and (2) at a later point, considerations of the actual biochemical nature of the kind of matter involved. Thus, the mathematics treats of the relations between objects, and the model is made to correspond more to the biological situation by taking into account the kinds of biochemical entities involved. We can then raise the question of how these mathematically oriented models can be useful in understanding biological systems.

ODUM: How do you get structure into mathematical models? Is this not the weakness of mathematical models? There is no way of indicating the structure of biological material.

FRIEDENBERG: Not necessarily. Physicists treat a structure by idealizing its characteristics into lines, surfaces, points, etc. Biologists could use the same technique.

ODUM: How do you treat different species, different things like mitochondria and nuclei, and so on?

FRIEDENBERG: In terms of their function.

ODUM: The whole secret is the relation between the structure and the function, and this cannot be done with a mathematical model.

FRIEDENBERG: If we idealize the structures and treat these idealized structures in terms such as the physicist would treat them, as surfaces and points, develop a mathematics around this to meet the function, and then come back to our system——

ODUM: This helps, but this is only a part of it.

POLLARD: The structure of the hydrogen atom is only described in mathematics, and it is the structure of the hydrogen atom that we are talking about.

ODUM: Yes, but Dr. Friedenberg mentioned the structure of a point, a molecule—what about the structure of a man?

McMULLEN: He is a series of points.

WATTS-TOBIN: I think it is worth pointing out how very much the structure of real things had to be idealized in many cases even to obtain results in mathematical physics.

ODUM: I am just asking.

HOFFMAN: On the same line, I do not think that argument is valid because the hydrogen atom is used as a very rough approximation for much larger, but just as simple, molecules. We could not talk about any one unit that we hope to explode up to a man to explain a man, but we may be able to make some kind of an approach—perhaps a "black box" approach to a system's interaction or perhaps a purely physical model based on known theories of physics of today—that might explain some of the movements within a small sector of a cell—for instance, a protein, an enzyme and its substrate interaction and movement. We start with those and go a little further.

YCAS: It seems we are getting a little away from the business of the conference here.

POLLARD: Not much. A little. It does not matter. We can soon come back.

FRIEDENBERG: For the record, I would like to say that I think this is a bona fide area of discussion in theoretical biology. I do not think there is any different kind of matter for the hydrogen atom than for the rest of matter, whether it be a man or a hydrogen atom.

POLLARD: I think I am going to interrupt this discussion. We are talking more about politics than we are about science in this business session. We have only a few more minutes to do it in and the question is, really, What factors would make a climate in which, let us be frank, we would gamble on the possibility that there would be something of value? I think this has to be admitted; it may yield nothing, just as this conference may yield nothing but some unusual friendships that are created and, perhaps, the cataloging in a single place of things that are found in scattered conditions. This may be all we will get, or it may not be; we do not know.

I think we have gotten a very good impression on quite a lot here. Let me ask a question. Is it the impression that support for a year or two years of individuals to do this type of theoretical biology is worthwhile? Is this a good thing? Should we ask for it? Is this something that the committee should recommend as part of our system? Or is it not?

LEIGH: Is it also possible to discuss the problem of setting up permanent chairs in theoretical biology?

POLLARD: Yes, it is. I would assume that if things such as this were successful initially, they would be followed by permanent chairs. I would assume so.

But let me ask a few questions here. Let me ask Dr. Pittendrigh. Suppose you were invited to a professorship at La Jolla in theoretical biology at $5000 more a year than you are getting now; would you go?

PITTENDRIGH: I feel really quite strongly that the biologist's situation at the moment is quite different from the physicist's. Suppose for instance, you had asked this question in 1950, before we had the Watson-Crick model; how far would you have gotten without the experimenting, without the idea? Advancing biology at the moment is still so utterly dependent on good experimental results, and I do not just mean doing just another experiment. But I really cannot

see for the most part that a person can go off for a year and "do" theoretical biology. He should be doing it all the time, but in the laboratory.

SAGER: Two problems are being confused—both of them, I think, in the area of theoretical biology. The easier one to deal with is the area in which there are already fairly well-formulated problems. For example, I came to this meeting with what I call a practical theoretical problem. It is loosely formulated but it needs much help in terms of model building and so forth. There is an experimental program in this area already.

But there is another area which I think is extremely exciting, but much less clear; namely, how to think along new lines and to be unfettered in one's thinking—in a sense, to get away from the laboratory simply in order to be able to think more freely.

Problem solving in this area is not handled by creating chairs in theoretical biology. It is certainly aided and abetted by having a free kind of place where people can get together and talk about some of these poorly formulated areas. On the other hand, much, perhaps all, problem solving occurs the way that Dr. Garfinkel was describing, simply by the work one does and the thinking one does by himself.

POLLARD: Let me make a statement on the other side. If I were asked whether I, as head of the Biophysics Department at Pennsylvania State University, would accept an invitation to serve in theoretical physics for the next two years on the assumption that now and again I might be interested in biology, I would accept it. So that while I think I have written already "Go slow here" because it does not look as though people would jump to take $20 000 jobs, I have an idea there are $20 000 vacancies that can be filled.

BAUTZ: I would think of it as a gamble, I must say, if I took the job for two years, because I do not know what is going to come out of it and I would rather like to think of a summer first.

DANIELLI: I think that a good deal of the hesitation about whether one should take a job like this arises from the fact that, practically speaking, there are not any properly trained theoretical biologists. There are people who are beginning to move into this field from different areas but who are naturally hesitant about the extent to which they would be successful. They feel they need a sheet anchor in the form of an experimental program. The physicist probably does not feel this way. He can move into theoretical physics, knowing that this has been a field of profound achievement for many years, and expect to do well if he has intuitions in that direction.

Therefore, I think that the most likely people to take up relatively long-term or permanent appointments in theoretical biology are those who have been trained in mathematics, or theoretical physics, or theoretical chemistry, who are accustomed to having confidence in the capacity of properly trained people to perform well in the theoretical field. Those who have been brought up as experimental biologists probably will move only gradually into this feeling of confidence. Insofar as we have a program of that sort, it probably ought to be directed more to bringing the mathematicians and the physicists and the chemists into theoretical biology than coaxing experimental biologists to let go their sheet anchor and become full-time theoretical biologists.

POLLARD: I think I am going to say that we have discussed the vital points. Dr. Jacobs, is this helpful to you?

JACOBS: Yes. I wish it were more definitive, but I think it is about as far as we can go under the circumstances.

QUIMBY: I would like to be definitive. I feel that we have been a bit out in space and should come back to Earth. First of all, $20 000 professorships may be hard to come by if the Federal Government is to be involved. NASA is not likely to set up "chairs" in theoretical biology.

Also, Dr. Pollard, I cannot see why anyone has to be uprooted from where he is and go to another place for one of these fellowships if there is, indeed, to be one. I do not see why Dr. Morowitz cannot stay where he is, why Dr. Pittendrigh cannot stay where he is, etc.

POLLARD: I do. If he has 15 people on PPLO alone in his laboratory, he will take $20 000 of that money and misspend it.

QUIMBY: We thought about that, too.

POLLARD: That is why the uprooting is necessary. We have to get him away from a laboratory.

QUIMBY: We do have in NASA predoctoral fellowships and postdoctoral associateships.

POLLARD: That is not the same.

QUIMBY: Perhaps not. May I change this subject? I am thinking of a paper which was written by a geologist who thought that his own discipline was an eclectic one. He thought that the purpose of science was to bring order out of chaos, to bring simplicity out of complexity. It seems to me that, if anything, this is what theoretical biology, if that is what we wish to call it, would try to do for the life sciences.

The advantages of getting away from the laboratory for unfettered thinking with fellow scientists are obvious. For a long time in this country, and probably throughout the world, we have had a more or less arbitrary separation of disciplines. This has been true in the universities where departments dominate the academic structure, and it is also true in the scientific societies. I think what is needed is to bring selected scientists with special knowledge from relevant or bordering disciplines together long enough for them to stimulate some new ideas and to lay their experimental activities to rest.

Perhaps this is the answer to Dr. Pittendrigh's question as to why we need theoretical biology.

PITTENDRIGH: That was not my question. I asked what it is.

QUIMBY: I thought you also asked why we need it.

PITTENDRIGH: No, I did not.

QUIMBY: Then may I ask the question of why we need theoretical biology? I do not care much about defining it. I think the reason we need it is because there are a number of unsolved problems, riddles, and mysteries in biology, which have existed for years. We describe them, measure them, and wring the last drop of water out of them if that is where we have made our reputations. But we do not understand them; we just marvel at them—bask in their complexity—and excuse our ignorance with the fact that the problems are complex. I am fully aware that biology and medicine have grossed a vast array of achievements without physics and very much mathematics. I am also aware that these achievements have been based upon mechanistic assumptions. This term intrinsically means physics and mathematics. I am inclined to ask for assistance from the mathematicians and the physical scientists in both experiment and theory. There seems little to lose by inventing a new field for modernizing modern biology. This statement in no way implies that careful detailed observation and descriptive biology should not continue.

McMULLEN: Will these experiments solve the problem, once again, of the origin of life and, if not, is it still legitimate to theorize about it as part of theoretical biology?

QUIMBY: Are you asking me?

McMULLEN: Anyone.

POLLARD: It is perfectly clear that we can theorize about anything.

McMULLEN: Is it interesting to do so? Is it worth a professorship to study the origin of life from a speculative point of view?

POLLARD: This is a somewhat different problem than is being discussed at the moment. I think our discussion concerns at the present time any theoretical biology; it does not make any difference what it is. I do not think I would want to recommend to NASA the establishing of a chair in the origin of life, necessarily. I would not hold it to that. After that, obviously, we have the theory of how the brain operates, the theory of pain sensation, and all of the things that are valid theories.

DANIELLI: Dr. Pollard, I think I would like to emphasize again that we cannot really predict with certainty what is the most profitable way of assisting in the development of theoretical biology at this time because, in fact, we have to work with the human material that is available. The only effective way of working with human beings is to find out what they are enthusiastic about and provide the means by which they can go ahead and do what they want. It does not matter in what area we are, provided we make progress.

Therefore, I feel that if we decide to put our funds in a summer institute, we must obtain the people who are enthusiastic about participating in a summer institute. There may be other methods which would use the money to greater advantage—I could not say. But I do not think we can fail, and I do not think we should worry about the relative efficiency of different methods of doing this job.

POLLARD: All right. I would like to thank you very much for the great generosity in helping in a real problem, and I am now going to declare the business meeting adjourned.

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